NEUROLOGICAL DISEASE AND THERAPY

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This book is dedicated to the brave individuals and their families who cope with multiple sclerosis and its uncertainties on a daily basis.
Preface

Approximately 400,000 individuals in the United States and two million people worldwide have multiple sclerosis.

Up until 1993, no drugs were available to alter the course of this often debilitating disease. Much has changed since that time. Over the last decade, six drugs have been approved in the United States for the treatment of multiple sclerosis and, fortunately, more are on the horizon. Although not curative, these drugs decrease the frequency and, in some instances, the severity of acute attacks, slow the rate of neurological deterioration, at least for the short term, and diminish the number of new lesions seen on magnetic resonance imaging studies. For the first time, patients can look forward to at least partial control of their illness and the likelihood that newer and better drugs will be available in the near future. Despite this progress, many questions still remain to be answered. Fundamental issues such as determining the cause of multiple sclerosis, defining the precise mechanism of tissue injury, and understanding ways to promote regeneration of myelin and axons need to be resolved before multiple sclerosis can be controlled or cured and, hopefully, a patient’s neurological disability can be reversed. Advances in molecular biology, genetics, chip technology, proteomics, nanotechnology, informatics, neuroimaging, and the availability of patient databases have provided the necessary tools for resolving these issues in a timely fashion.

The fourth edition of the *Handbook of Multiple Sclerosis* updates the reader as to current knowledge about basic and clinical aspects of multiple sclerosis, therapeutic advances, and prospects for future research directives. As with previous editions, the fourth edition is meant to be a comprehensive reference book for practitioners, scientists, students, and patients and their families. I am very grateful to the contributors, who are world leaders in multiple sclerosis research and treatments.

*Stuart D. Cook*
Acknowledgment

The editor appreciates the work and effort provided by Ms. Mary Perez in making this edition of the *Handbook of Multiple Sclerosis* possible.
Contents

Preface . . . . v
Acknowledgment . . . . vii
Contributors . . . . xvii

PART I: ETIOPATHOGENESIS

1. Etiopathogenesis and Epidemiology: Clues to Etiology ............... 1
   William Pryse-Phillips and Scott Sloka
   Introduction . . . . 1
   Familial Factors and Genetic Susceptibility . . . . 2
   Prevalence and Incidence Studies . . . . 6
   Intercurrent Factors with Possible Association . . . . 13
   Natural History and Clinical Variability . . . . 22
   Clues to Etiology . . . . 25
   References . . . . 30

2. Genetics: Susceptibility and Expressivity ............................ 41
   Thomas Masterman and Jan Hillert
   Susceptibility . . . . 41
   Expressivity . . . . 49
   Prospects . . . . 50
   References . . . . 56

3. Evidence for an Infectious Etiology of Multiple Sclerosis .................. 65
   Stuart D. Cook
   Introduction . . . . 65
   Historical Perspective . . . . 65
   Evidence for an Infectious Etiology . . . . 67
   Possible Mechanisms of Virus-Induced Demyelination . . . . 70
   Candidate Agents in MS . . . . 73
   Conclusion . . . . 85
   References . . . . 85
4. Multiple Sclerosis: An Autoimmune Disease of the Central Nervous System? ........................................ 95
   John R. Rinker II, Robert T. Naismith, and Anne H. Cross
   Introduction .... 95
   Epidemiology .... 96
   Pathology Suggests an Autoimmune Etiology .... 96
   Genetic Evidence Supporting Autoimmunity in MS .... 97
   Evidence for T-Cell Mediated Autoimmunity in MS .... 98
   Humoral Immunity as Indirect Evidence for Autoimmunity in MS .... 101
   Response to Immunosuppressive Therapies Suggests an Autoimmune Etiology .... 103
   The Autoimmune Hypothesis Is Supported by Animal Models .... 103
   Summary .... 104
   References .... 105

PART II: CLINICAL–PATHOLOGIC CHARACTERISTICS
5. Pathology: What May It Tell Us? .................................. 113
   Claudia F. Lucchinetti and Joseph E. Parisi
   Introduction .... 113
   How Does Stage of Demyelinating Activity Relate to Clinical Phase of the Disease? .... 114
   What Is the Pathogenic Role of Inflammation in MS? .... 119
   What Is the Fate of the Oligodendrocyte and Extent of Remyelination in MS Lesions? .... 121
   Is There Evidence for Pathologic Heterogeneity in MS? .... 123
   Does Pathologic Heterogeneity Reflect Pathogenic Heterogeneity in MS? .... 128
   What Is the Substrate of Irreversible Disability in MS? .... 130
   The Inflammatory Demyelination/Neurodegeneration Paradox .... 130
   What Is the Spectrum of Idiopathic Inflammatory Demyelinating Diseases? .... 136
   What Do New Molecular Studies Tell Us About the MS Lesion? .... 141
   Conclusion .... 143
   References .... 143
6. Clinical Features ........................................ 153
   Aaron E. Miller
   Introduction . . . 153
   Diagnosis . . . 153
   Age of Onset . . . 158
   Clinical Manifestations . . . 158
   Course . . . 166
   Pregnancy . . . 168
   References . . . 169

7. MRI Techniques in Multiple Sclerosis: Role in Diagnosis,
   Pathophysiology, and Therapy ........................... 179
   Massimo Filippi, Federica Agosta, Beatrice Benedetti, and
   Maria A. Rocca
   Introduction . . . 179
   A Brief Review of Basic Aspects of Nonconventional MRI
   Techniques . . . 182
   The Role of MRI in the Diagnosis and Prognosis
   of MS . . . 185
   The Role of MRI in Understanding MS
   Pathophysiology . . . 194
   MRI in Monitoring Treatment Efficacy in
   MS Trials . . . 204
   Conclusions . . . 207
   References . . . 208

8. Multiple Sclerosis Biomarkers ............................ 223
   P. K. Coyle
   Introduction . . . 223
   Potential Biomarkers . . . 226
   Neuroimaging . . . 235
   Conclusion . . . 236
   References . . . 236

9. Evoked Potentials ..................................... 243
   Marc R. Nuwer
   Introduction . . . 243
   Visual Evoked Potentials . . . 244
   Brainstem Auditory Evoked Potentials . . . 247
   Somatosensory Evoked Potentials . . . 249
   Motor EPs . . . 252
   Event-Related Potentials . . . 253
   Multimodality EP Testing . . . 253
   Use of EPs in MS Therapeutic Trials . . . 256
   References . . . 260
PART III: THERAPIES—CURRENT AND FUTURE

10. Managing the Symptoms of Multiple Sclerosis ................. 271
   Randall T. Schapiro
   Introduction . . . . 271
   Fatigue . . . . 271
   Spasticity . . . . 272
   Weakness . . . . 273
   Urinary Dysfunction . . . . 274
   Bowel Dysfunction . . . . 274
   Sexual Dysfunction . . . . 275
   Pain . . . . 275
   Tremor . . . . 276
   Visual Dysfunction . . . . 276
   Paroxysmal Spasms . . . . 277
   Pathological Laughing/Crying . . . . 277
   Depression . . . . 277
   Conclusion . . . . 277
   References . . . . 278

11. Rehabilitation: Its Role in Multiple Sclerosis ................. 281
   George H. Kraft and Anjali N. Shah
   Introduction . . . . 281
   Fatigue . . . . 282
   Weakness and Spasticity . . . . 284
   Body Cooling . . . . 289
   Ataxia and Tremor . . . . 291
   Sensory Loss and Pain . . . . 292
   Depression . . . . 293
   Cognitive Impairment . . . . 293
   General Fitness . . . . 294
   Assistive Technology . . . . 295
   Vocational Issues . . . . 296
   Conclusion . . . . 296
   References . . . . 297

12. Acute Treatments ............................................. 301
   Brian G. Weinshenker and Nima Mowzoon
   Introduction . . . . 301
   Treatment with Corticosteroids . . . . 304
   Intravenous Immunoglobulin . . . . 306
   Therapeutic Plasma Exchange . . . . 308
   Mitoxantrone . . . . 310
   Cyclophosphamide . . . . 311
   Conclusions . . . . 312
   References . . . . 313
13. Treatment of the Clinically Isolated Syndromes ......................... 317
   Giancarlo Comi
   Introduction .... 317
   Rationale for Early Treatment .... 317
   Conclusion .... 327
   References .... 327

14. The Use of Interferon Beta in the Treatment of Multiple Sclerosis ......................... 333
   Douglas S. Goodin
   Introduction .... 333
   Biological Consequences of IFNβ Administration .... 335
   Assessing the Clinical and MRI Effects of IFNβ in MS Patients .... 336
   Conclusions .... 346
   References .... 347

15. Glatiramer Acetate (Copaxone) ................................ 351
   Yang Mao-Draayer and Hillel S. Panitch
   Introduction .... 351
   Clinical Studies of GA .... 351
   Immunological Activity of GA .... 361
   The Place of GA in MS Therapy .... 365
   Conclusion .... 366
   References .... 366

16. Mitoxantrone ..................................................... 373
   Oliver Neuhaus, Bernd C. Kieseier, and Hans-Peter Hartung
   Introduction .... 373
   Evidence Leading to the Approval of Mitoxantrone for Use in Multiple Sclerosis .... 373
   Current Clinical Aspects of Mitoxantrone .... 376
   Putative Mechanisms of Action of Mitoxantrone .... 379
   Conclusions .... 381
   References .... 381

17. Monoclonal Antibodies, T-Cell Receptors, and T-Cell Vaccines .................................. 385
   Flavia Nelson and Jerry S. Wolinsky
   Introduction .... 385
   Monoclonal Antibodies .... 385
   T-Cell Vaccines .... 398
   Conclusion .... 401
   References .... 402
18. Immune Therapy for Multiple Sclerosis: Altered Peptide Ligands and Statins ........................................ 409
   Fu-Dong Shi, Denise I. Campagnolo, and Timothy L. Vollmer
   Introduction . . . . 409
   Is the Use of APLs Still a Viable Approach for Treatment of MS? . . . . 409
   Are Statins a Treatment Option for MS? . . . . 413
   References . . . . 419

19. Immunosuppression ............................................. 423
   Harold Atkins and Mark Freedman
   Introduction . . . . 423
   Cyclophosphamide . . . . 424
   Complete Immunoablation and Autologous Stem Cell Transplantation . . . . 426
   Transplant Studies in MS . . . . 427
   Stem Cell Transplantation . . . . 429
   MS Outcomes Following Transplantation . . . . 433
   Patient Selection . . . . 434
   Future Directions—Beyond Cytotoxic Immunosuppression . . . . 434
   Future Directions—Beyond Repair of the Immune System . . . . 435
   Conclusion . . . . 435
   References . . . . 435

20. Combination Therapy in Multiple Sclerosis .................. 443
   Mark J. Tullman and Fred D. Lublin
   Introduction . . . . 443
   Selecting Agents for Combination Therapy . . . . 443
   IFNβ and GA . . . . 445
   IFN, Methylprednisolone, and Methotrexate . . . . 447
   Mitoxantrone and Methylprednisolone . . . . 448
   Conclusions . . . . 449
   References . . . . 450

21. Regeneration Strategies for Multiple Sclerosis ............... 453
   Arthur E. Warrington and Moses Rodriguez
   Introduction . . . . 453
   Remyelination as a Normal Reparative Response . . . . 454
   Present Treatments for MS Target Inflammation, Not Repair . . . . 454
   Inflammation Hinders as well as Facilitates CNS Repair . . . . 455
   Growth Factors for MS Lesion Repair and Regeneration . . . . 456
Cell Transplantation for MS Lesion Repair and Regeneration . . . 457
Pathogenic Antibodies Directed Against CNS Antigens . . . 459
Reparative Antibodies Directed Against CNS Antigens . . . 459
Glatiramer Acetate, an Established Treatment for MS, May Act via a Humoral Immune Response . . . 463
Mechanism of Antibody-Mediated CNS Repair . . . 464
Remyelination Promoting mAbs Target the Damaged CNS . . . 466
The Challenge of Balancing Inflammation for Regeneration . . . 467
References . . . 467

22. Axonal Injury in Multiple Sclerosis .......................... 477
Gerson A. Criste and Bruce D. Trapp
Introduction . . . 477
Axonal Pathology in MS Lesions . . . 477
Mechanism of Axonal Injury in MS . . . 481
Strategies for Axonal Protection . . . 486
Surrogate Markers of Axonal Loss . . . 489
Clinical Implications . . . 495
Conclusion . . . 496
References . . . 497

Index . . . 507
Contributors

Federica Agosta  Neuroimaging Research Unit, Department of Neurology, Scientific Institute and University Ospedale San Raffaele, Milan, Italy

Harold Atkins  Ottawa Hospital Blood and Marrow Transplant Program, Ottawa, Ontario, Canada

Beatrice Benedetti  Neuroimaging Research Unit, Department of Neurology, Scientific Institute and University Ospedale San Raffaele, Milan, Italy

Denise I. Campagnolo  Barrow Neurological Institute, St. Joseph’s Hospital and Medical Center, Phoenix, Arizona, U.S.A.

Giancarlo Comi  Department of Neurology and Clinical Neurophysiology, Vita-Salute University, San Raffaele Scientific Institute, Milan, Italy

Stuart D. Cook  Department of Neurology/Neurosciences, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey, U.S.A.

P. K. Coyle  Department of Neurology, School of Medicine, State University of New York at Stony Brook, Stony Brook, New York, U.S.A.

Gerson A. Criste  Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio, U.S.A.

Anne H. Cross  Department of Neurology and Neurosurgery, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

Massimo Filippi  Neuroimaging Research Unit, Department of Neurology, Scientific Institute and University Ospedale San Raffaele, Milan, Italy

Mark Freedman  Ottawa Hospital Multiple Sclerosis Clinic, Ottawa, Ontario, Canada

Douglas S. Goodin  Department of Neurology, University of California, Ft. Miley Veterans Administration Hospital, San Francisco, California, U.S.A.
Hans-Peter Hartung  Department of Neurology, Heinrich Heine University, Düsseldorf, Germany

Jan Hillert  Division of Neurology, Department of Clinical Neuroscience, Karolinska Institutet, Karolinska University Hospital, Huddinge, Stockholm, Sweden

Bernd C. Kieseier  Department of Neurology, Heinrich Heine University, Düsseldorf, Germany

George H. Kraft  Department of Rehabilitation Medicine and Neurology, University of Washington, Seattle, Washington, U.S.A.

Fred D. Lublin  Corinne Goldsmith Dickinson Center for Multiple Sclerosis, Mount Sinai School of Medicine, New York, New York, U.S.A.

Claudia F. Lucchinetti  Department of Neurology, Mayo Clinic, Rochester, Minnesota, U.S.A.

Yang Mao-Draayer  Department of Neurology, University of Vermont College of Medicine, Burlington, Vermont, U.S.A.

Thomas Masterman  Division of Neurology, Department of Clinical Neuroscience, Karolinska Institutet, Karolinska University Hospital, Huddinge, Stockholm, Sweden

Aaron E. Miller  Mount Sinai School of Medicine, New York, New York, U.S.A.

Nima Mowzoon  Department of Neurology, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

Robert T. Naismith  Department of Neurology and Neurosurgery, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

Flavia Nelson  Department of Neurology, The University of Texas Health Science Center, Houston, Texas, U.S.A.

Oliver Neuhaus  Department of Neurology, Heinrich Heine University, Düsseldorf, Germany

Marc R. Nuwer  Department of Neurology, UCLA School of Medicine, and Department of Clinical Neurophysiology, UCLA Medical Center, Los Angeles, California, U.S.A.

Hillel S. Panitch  Department of Neurology, University of Vermont College of Medicine, Burlington, Vermont, U.S.A.

Joseph E. Parisi  Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, U.S.A.
Contributors

**William Pryse-Phillips**  Division of Neurology, Memorial University of Newfoundland and Health Science Center, St. John’s, Newfoundland and Labrador, Canada

**John R. Rinker II**  Department of Neurology and Neurosurgery, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

**Maria A. Rocca**  Neuroimaging Research Unit, Department of Neurology, Scientific Institute and University Ospedale San Raffaele, Milan, Italy

**Moses Rodriguez**  Departments of Neurology and Immunology, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

**Randall T. Schapiro**  The Schapiro Center for Multiple Sclerosis, Minneapolis Clinic of Neurology, and University of Minnesota, Minneapolis, Minnesota, U.S.A.

**Anjali N. Shah**  Multiple Sclerosis Program, University of Texas Southwestern Medical Center, Dallas, Texas, U.S.A.

**Fu-Dong Shi**  Barrow Neurological Institute, St. Joseph’s Hospital and Medical Center, Phoenix, Arizona, U.S.A.

**Scott Sloka**  Division of Neurology, Memorial University of Newfoundland and Health Science Center, St. John’s, Newfoundland and Labrador, Canada

**Bruce D. Trapp**  Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio, U.S.A.

**Mark J. Tullman**  Corinne Goldsmith Dickinson Center for Multiple Sclerosis, Mount Sinai School of Medicine, New York, New York, U.S.A.

**Timothy L. Vollmer**  Barrow Neurological Institute, St. Joseph’s Hospital and Medical Center, Phoenix, Arizona, U.S.A.

**Arthur E. Warrington**  Departments of Neurology and Immunology, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

**Brian G. Weinshenker**  Department of Neurology, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

**Jerry S. Wolinsky**  Department of Neurology, The University of Texas Health Science Center, Houston, Texas, U.S.A.
INTRODUCTION

In the last edition of this handbook, this chapter concluded with the statement that epidemiology is inferential—its role is to provide etiologic clues, but it cannot prove nor refute causality. Multiple sclerosis (MS) is a complex trait that appears to be determined by both genetic and environmental factors. It exhibits a changing incidence over time in an uneven geographic distribution. The evidence of these spatial and temporal trends from migrant studies affirms the etiological relevance of environmental factors, although their nature remains mysterious and is undoubtedly complex. A best summarizing guess at the causality of the disease was proffered, supposing that all MS patients possess one of the range of genotypes that confer susceptibility, that different genotypes are associated with different phenotypes, and that about one-third of those susceptible will develop the disease while the remaining two-thirds will not—either because they possess inhibitory or protective genes or because they do not come into contact with the necessary triggering factors in their internal or external environments.

The task for classic, inferential epidemiology is yet to standardize case findings, diagnostic criteria, and other methodological considerations to elucidate the contributory factors. Over the last five years, further epidemiological data that help in the understanding of the nature and cause(s) of MS have been published; a selection of these are reviewed here. During this period, Rosati (1) has noted that the influence of genetic factors in MS acquisition has been suggested by its rarity among certain races and by the relatively high risk among others. Such findings clearly indicate that the different susceptibilities of distinct racial and ethnic groups contribute to determining the uneven geographic distribution of the disease. The distribution of MS in Europe, however, shows many exceptions to the enigmatic north–south gradient and requires more explanation than a simple prevalence–latitude relationship.

In order to present an organized discussion of current clues to etiology, we have constructed a framework for the natural history of MS (Fig. 1) in the context of the various epidemiological observations toward the search for etiology thus far.
A natural history of the disease would begin with genetic or familial factors conferring susceptibility or protection to an individual at conception. It is likely that multiple genetic factors contribute to such susceptibility and that different combinations of such factors affect the length of the induction period (from the first disease trigger to a sufficient disease trigger), but the number and nature of the environmental exposures required for disease initiation or clinical progression remain speculative. The environmental contribution to disease initiation may occur either in utero or after birth. Different environmental factors (e.g., an exposure to a disease-triggering event) and multiple exposures may be necessary, again depending on the genetic susceptibility of the individual.

Susceptibility to disease induction in an individual may depend on a critical age of susceptibility or on a specific milestone (e.g., the evolution to puberty). This age of susceptibility may be variable, given selected genetic factors. Following this critical age, a latency period ensues before the clinical expression of the disease. To add further complexity to the model, different clinical forms of MS have been described, with different clinical courses, natural histories, ages of onset, rates of progression, and male-to-female ratios. Therefore, any etiological hypotheses drawn from epidemiological observations should be made in the context of the specific disease subtype.

**FAMILIAL FACTORS AND GENETIC SUSCEPTIBILITY**

The genetics of MS are discussed in a subsequent chapter. Here, we briefly address recent observations on the epidemiology of the genetics of MS and how this pertains to the elucidation of etiology (Fig. 2).

**Family Studies**

Both specific genetic markers (e.g., the HLA-DR2 haplotype on chromosome 6) (2) and twin studies have inferred a genetic susceptibility. Concordance rates for MS,
among monozygotic twins, range between 31% and 40%, whereas those among fraternal and nontwin siblings are between 3% and 5%, (3,4) demonstrating a significant increase in relative risk with increasing genetic similarity. Because prevalence rates of MS among nonbiological siblings adopted into a family are similar to those found in the general population and are significantly less for biological relatives, familial aggregation of MS is obviously important (3). But the mode of disease transmission shows neither classical recessive nor dominant traits (5) and without a cost-effective means of population screening, disease penetrance is difficult to estimate.

Within an affected sibship, the initial clinical presentations differ but ultimate concordance for disease course (disability, progression) is likely (6). Familial recurrence rates of 1.9% to 4.7% have been found (7–9). The risk ratio of first-degree relatives compared with the general population was 31 times in one study (9). The risk is highest overall for siblings (4.8%), children (2.3%), and parents (1.3%), with lower rates in second-degree (0.7%) and third-degree (1.8%) relatives. Recurrence is highest for monozygotic twins (8,10). The risk for siblings is influenced by the age at onset and possibly by gender (9); male gender of the probands, female gender of the relatives, and the number of affected relatives in the family significantly increase the risk of MS in relatives (8). There is also a borderline significant interaction between the sex and age at onset of the proband; early age at onset influences sibs’ risk only if the proband is female (9). Times to disability do not differ significantly when sporadic, familial and familial subgroups are compared, although the parent of origin may influence disability and disease course as well as increase the risk to additional offspring within the same family (11).

**Regional Population Studies**

The variation of prevalences within a localized geographical region has been studied and may act as a compass to etiology. A migration model for the epidemiology of MS in Newfoundland and Labrador was recently constructed, which accounts for both country of origin (a possible genetic contribution) and latitude (a potential environmental contribution) (12). This model, based on known migration patterns in a region with a strong founder effect and low intraregional migration, demonstrates that at least a portion of Newfoundland’s population prevalence may be accounted for by their country of origin, a pattern that is further refined by the presumed environmental component of latitude.

The developing field of small area analysis has aided in the search for disease clusters in MS. A clustering pattern of prevalent cases (and a west-to-east gradient) has been found in some southwestern Sardinian communes, based on geographic

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**Figure 2**  Factors contributing to genetic susceptibility to multiple sclerosis explored in recent epidemiological studies.
distribution by both current prevalence and residence at the age of 5 to 15 years. Such clustering was found in a common linguistic area, while another adjacent (but genetically distinct) population showed lower figures (13). The authors hypothesized that a widely and evenly spread environmental agent may trigger disease in those subgroups of individuals who are genetically more susceptible.

Comment

The occurrence of regional clustering on a defined prevalence day does not distinguish between genetic and environmental influences; the latter would be differentiated by peaks of incidence within a defined geographical area in unrelated people, as in Key West and Sitka, AL (discussed in previous editions of this book). Temporal, as well as spatial cluster analyses may further contribute to the search for environmental causes within a genetically homogeneous population.

Racial Factors

Over the last five years, papers from all inhabited continents have documented the prevalence or incidence and the clinical phenotypes of MS. Thus, among Bantu African Kenyans, MS incidence rates are increasing (14). MS in Japan has a higher age at onset and a higher female-to-male ratio than conventional MS, and opticospinal MS is unusually frequent, although in Japanese people born after the 1960s, the ratio of conventional to opticospinal MS has rapidly increased (15) at the same time as there has been increased contact between the Japanese and Western peoples. Of interest is the observation that conventional MS in Japanese people is, like MS in white people, associated with HLA-DRB1*1501, whereas opticospinal MS is associated with HLA-DPB1*0501 (16).

In a retrospective study from Manitoba, Canada, seven aboriginals were identified as having MS, giving an unusually low period prevalence rate of 40/10^5. As in other eastern-derived populations, the clinical features included phenotypes with aggressive disease courses and more frequent involvement of optic nerves and spinal cord compared with nonaboriginal patients. Aboriginals of Algonkian background also seem to be at increased risk for the aggressive type of MS, independent of those HLA alleles known to be associated with MS (17).

Conjugal Rates

While Hawkes (18) suggested that family, conjugal pair, twin, and adoption studies are compatible with an infectious cause of MS if this is sexually transmitted, Ebers et al. (3) considered that the low risk for the spouses of MS patients but the high risk in their offspring indicates that the familial risk reflects genetic determination (7), contradicting the supposition that MS is a sexually transmissible disease, at least in the marriage-age group. However, MS may still be interpersonally transmitted during a period of susceptibility that is earlier than the age of marriage, bearing in mind those close, asexual physical contacts that children have (more often with their mothers than with their fathers).

Birth Month

The onset of optic neuritis (ON) and of MS in the northern hemisphere occurs most commonly in spring and least often in winter (19). Seasonal birth studies in MS,
amyotrophic lateral sclerosis (ALS), and possibly Parkinson disease also show an excess of spring births (20). However, a Sicilian population of MS patients has shown a highly significant excess of births between June and November (21). Studies of season of birth and risk of MS have been scanty and controversial until the recent demonstration (22) of a significant increase in the numbers of MS patients born in May compared with the numbers born in November, in the northern hemisphere. There is no obvious reason why differing nine-month calendar periods of intrauterine development should influence MS incidence two decades or so later; speculations about incident radiation (e.g., UV radiation generates vitamin D which modulates helper T2 lymphocytes to counterbalance the activity of helper T1 lymphocytes) can be constructed but are as yet unsubstantiated. Should future studies reveal that the more frequent months of birth are inverted in the southern hemisphere, the construction and testing of theories of causation based on these findings will be of paramount importance.

**Mortality Studies**

MS reduces life expectancy. Among 1614 Finnish MS patients, survival rates 40 years after diagnosis were 64% for MS-related deaths (c.f. 53% for all deaths). The proportions of violent deaths and neoplasms were higher in the general population, but that of cardiovascular deaths was relatively low (23).

In Canada, over the 30 years from 1965, the highest average annual MS mortality rates were in Quebec (4.4/10^5) and Ontario (3.9), while the western provinces had an intermediate rate (2.1) and the Atlantic provinces the lowest rate (1.2). The overall average annual MS mortality rates in Canada have fluctuated during the past 30 years, but there is no apparent relationship between prevalence and mortality rates among the Canadian provinces (24).

In a Danish study of 9881 patients, of whom 4254 had died before the end of follow-up, the median survival time from onset was approximately 10 years shorter for MS patients than for the age-matched general population, and MS was associated with an almost threefold increase in the risk for death (25). MS patients also had excess mortality rates from other diseases, except cancer, and from accidents and suicide. On the brighter side, the 10-year excess mortality rate was almost halved in comparison with that of the middle of the 20th century.

In a large study of U.S. veterans (26), median survival times from onset of MS were 43 years for white females, 30 years for black males, and 34 years for white males, whereas crude 50-year survival rates were 31.5% for white females, 21.5% for black males, and 16.6% for white males; only the figures for white females and white males differed significantly. Standardized mortality ratios utilizing national U.S. data (for 1956–1996) showed a similar marked excess for all three race–sex groups of MS cases but with a decreasing excess over time. Relative survival rates, comparing the survival of veterans with MS and those without, differed significantly by socioeconomic class but not by sex–race group, suggesting that the significant difference in survival between male and female MS cases results in part from gender rather than disease.

Tassinari (27) computed standardized mortality ratios in Italy for the period 1974–1993. Age-adjusted rates per million inhabitants were 4.1 for males and 5.0 for the period females. Northern Italian regions had higher MS mortality rates than central and southern regions and Sicily, particularly for females. Over these years, a statistically significant increase was seen for both males (+2.14%) and females (+3.09%) in the south and Sicily.
During a 10-year observation period, 21% of MS patients died but only 70% of them had an entry denoting MS in the death statistics (28). Because only a few papers provide details of the causes of death, and because of the notorious unreliability of death certificates, calculations of incidence or prevalence rates on the basis of death certificates appear unprofitable (29) and will not be discussed here. Although the quality of the data and their interpretation are open to question, they still remind us that depression, suicide, infections, and motor impairments in MS patients constrain living and truncate life.

PREVALENCE AND INCIDENCE STUDIES

Incidence and prevalence rates continue to be reported from all parts of the world, but one is cautioned by the words of Rosati (1). “The comparison of prevalence studies carried out in different areas and times is made difficult by the variability in surveyed population sizes, age structures, ethnic origins and composition, and the difficult quantification of numerators, especially regarding the recognition of benign and very early cases. Additionally, complete case ascertainment depends on access to medical care, local medical expertise, numbers of neurologists, accessibility and availability of new diagnostic procedures, the degree of public awareness about MS, and the investigators’ zeal and resources.”

A summary of studies published recently is provided in Table 1. These studies have wide variation in methodology, but two conclusions can be drawn from the aggregate. First, although a general latitude gradient may still be perceived (Fig. 3) there are important differences in the rates reported at similar latitudes, possibly explicable in terms of racial or ethnic differences; and second, the incidence and prevalence rates reported have increased whenever a study was repeated.

It is regrettable that any comparison of prevalence between published regional studies has limited validity due to differences in age distribution, ethnic composition, and case ascertainment, as well as to changes in prevalence over time. Recently presented data have cast doubt upon the reliability of all the estimates published to date. The regional distribution of MS (Beck C, personal communication) showed prevalence rates between 180 and 350/10^5 in five transnational geographic regions in a population health survey of 131,535 Canadians in 2000/2001. The overall weighted estimate of MS prevalence in Canada was 240/10^5. Regional weighted prevalences ranged from 180 in Quebec to 350/10^5 in the Atlantic provinces. The odds of having MS in the Prairies and Atlantic regions were significantly elevated when compared with other regions. While the results were based upon self-report in a random telephone interview and not clinically confirmed, figures from the Alberta healthcare agency supported these estimates, doubling or tripling the prevalence rates reported hitherto in Canada. If this methodology is sound, the conclusions drawn from all previous studies must be questioned, as they would have been based upon findings in a limited sample.

Variation with Latitude

The correlation of prevalence with latitude is often quoted and still holds in the presence of updated prevalence studies (74). In Australia, the strong correlation with latitude (the disease becoming increasingly prevalent with increasing southern
<table>
<thead>
<tr>
<th>Year</th>
<th>Place</th>
<th>Latitude (approx.)</th>
<th>Prevalence/10^5</th>
<th>References</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>North Norway</td>
<td>65–70° N</td>
<td>93</td>
<td>Gronlie et al. (30)</td>
<td>New cases now found in the Sami population</td>
</tr>
<tr>
<td>Bulgaria</td>
<td></td>
<td>42° N</td>
<td>44.9</td>
<td>Milanov et al. (31)</td>
<td>Europoids</td>
</tr>
<tr>
<td>Bulgaria</td>
<td></td>
<td>42° N</td>
<td>19.1</td>
<td>Milanov et al. (31)</td>
<td>Gypsies</td>
</tr>
<tr>
<td>North Spain</td>
<td></td>
<td>41° N</td>
<td>58.3</td>
<td>Tola et al. (32)</td>
<td></td>
</tr>
<tr>
<td>1990</td>
<td>North Sweden</td>
<td>65–70° N</td>
<td>125</td>
<td>Sundstrom et al. (33)</td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td></td>
<td>47° N</td>
<td>98.5</td>
<td>Baumhackl et al. (34)</td>
<td></td>
</tr>
<tr>
<td>Hungary</td>
<td></td>
<td>47° N</td>
<td>62</td>
<td>Bencsik et al. (35)</td>
<td></td>
</tr>
<tr>
<td>Martinique</td>
<td></td>
<td>15° N</td>
<td>14.3</td>
<td>Merle et al. (36)</td>
<td>CDMS; French Afro-Caribbeans</td>
</tr>
<tr>
<td>Sao Paulo, Brazil</td>
<td></td>
<td>22° S</td>
<td>15</td>
<td>Callegaro et al. (37)</td>
<td>3×1990 rate</td>
</tr>
<tr>
<td>Menorca</td>
<td></td>
<td>40° N</td>
<td>68.6</td>
<td>Casquero et al. (38)</td>
<td>CDMS, CPMS</td>
</tr>
<tr>
<td>1995</td>
<td>Oslo, Norway</td>
<td>60° N</td>
<td>120.4</td>
<td>Celius et al. (39)</td>
<td>CDMS</td>
</tr>
<tr>
<td>North New Zealand</td>
<td></td>
<td>37° S</td>
<td>50</td>
<td>Chancellor et al. (40)</td>
<td>Repeated, increased, possibly due to better case ascertainment, longer life expectancy, a different diagnostic criteria, and changes in local population composition</td>
</tr>
<tr>
<td>1999</td>
<td>Malta</td>
<td>34° N</td>
<td>13.2</td>
<td>Dean et al. (41)</td>
<td>Repeated, still low; increase may be due to a longer expectation of life and the diagnosis is now made earlier</td>
</tr>
<tr>
<td>Tayside, Scotland</td>
<td></td>
<td>57° N</td>
<td>184</td>
<td>Forbes et al. (42)</td>
<td></td>
</tr>
<tr>
<td>North United Kingdom</td>
<td></td>
<td>54–56° N</td>
<td>180</td>
<td>Forbes and Swingler (43)</td>
<td></td>
</tr>
</tbody>
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(Continued)
<table>
<thead>
<tr>
<th>Year</th>
<th>Place</th>
<th>Latitude (approx.)</th>
<th>Prevalence/10^5</th>
<th>References</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>South United Kingdom</td>
<td>50–53° N</td>
<td>&lt;160</td>
<td>Forbes and Swingler (43)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leeds, United Kingdom</td>
<td>54° N</td>
<td>108.7</td>
<td>Ford et al. (44)</td>
<td>10% increase from 1996. Repeated, incidence increased but less than other published figures</td>
</tr>
<tr>
<td></td>
<td>Marina Alta, Spain</td>
<td>42° N</td>
<td>40.3</td>
<td>Garcia-Gallego and Morera-Guitart (45)</td>
<td>CDMS</td>
</tr>
<tr>
<td></td>
<td>Moscow, Russia</td>
<td>56° N</td>
<td>40.8</td>
<td>Gusev et al. (46)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Orel, Russia</td>
<td>52° N</td>
<td>64.8</td>
<td>Gusev et al. (46)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Germany</td>
<td>47–54° N</td>
<td>149.1</td>
<td>Hein and Hopfenmuller (47)</td>
<td>Repeated, similar prevalences</td>
</tr>
<tr>
<td></td>
<td>Canary Islands, Spain</td>
<td>28° N</td>
<td>42</td>
<td>Hernandez et al. (48)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hokkaido, Japan</td>
<td>44° N</td>
<td>8.6</td>
<td>Houzen et al. (49)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>North Japan</td>
<td>39–41° N</td>
<td>10.2</td>
<td>Itoh et al. (50)</td>
<td>4×1975 rate, but it is uncertain whether this apparent increase is real or reflects better ascertainment</td>
</tr>
<tr>
<td>1998</td>
<td>Faroes</td>
<td>62° N</td>
<td>66</td>
<td>Kurtzke and Heltberg (51)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hong Kong</td>
<td>22° N</td>
<td>0.77</td>
<td>Lau et al. (52)</td>
<td>Increasing since the 1980s</td>
</tr>
<tr>
<td></td>
<td>Alcoi, Spain</td>
<td>38° N</td>
<td>41</td>
<td>Mallada-Frechin et al. (53)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sardinia</td>
<td>40° N</td>
<td>157</td>
<td>Montomoli et al. (54)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>North Sardinia</td>
<td>41° N</td>
<td>149.7</td>
<td>Pugliatti et al. (55)</td>
<td>Onset-adjusted</td>
</tr>
<tr>
<td></td>
<td>North Sardinia</td>
<td>41° N</td>
<td>144.4</td>
<td>Pugliatti et al. (55)</td>
<td>Incidence 4.9 (was 2.0 before 1972). Increased, repeated, due to both case...</td>
</tr>
<tr>
<td>Year</td>
<td>Location</td>
<td>Latitude</td>
<td>Incidence</td>
<td>References</td>
<td>Notes</td>
</tr>
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<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>1993–1997</td>
<td>North Sardinia</td>
<td>41° N</td>
<td>144.4</td>
<td>Pugiatti et al. (13)</td>
<td>Ascertainment and true increase when compared with other locations</td>
</tr>
<tr>
<td></td>
<td>Central Sardinia</td>
<td>40° N</td>
<td>157</td>
<td>Montomoli et al. (9)</td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td>Dijon, France</td>
<td>47° N</td>
<td></td>
<td>Moreau et al. (56)</td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td>North Sardinia</td>
<td>41° N</td>
<td></td>
<td>Incidence 8.2</td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td>Central Sardinia</td>
<td>40° N</td>
<td></td>
<td>Incidence 6.1</td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td>Dijon, France</td>
<td>47° N</td>
<td></td>
<td>Incidence 5.5</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>Devon, United Kingdom</td>
<td>51° N</td>
<td>118</td>
<td>Fox et al. (57)</td>
<td>CDMS, CPMS McD criteria</td>
</tr>
<tr>
<td>1986</td>
<td>NW Poland</td>
<td>54° N</td>
<td>110.5</td>
<td>Potemkowski (58)</td>
<td>Peaks and clustering</td>
</tr>
<tr>
<td>1996</td>
<td>ACT Australia</td>
<td>35° S</td>
<td>49.5</td>
<td>Simmons et al. (59)</td>
<td>Both genders. CDMS</td>
</tr>
<tr>
<td>2000</td>
<td>Minnesota, U.S.A.</td>
<td>45°-47° N</td>
<td>177</td>
<td>Mayr et al. (60)</td>
<td>Stable for 20 yrs. Incidence 7.5</td>
</tr>
<tr>
<td>1994</td>
<td>U.S.A.</td>
<td>85</td>
<td></td>
<td>Noonan et al. (61)</td>
<td>50% increase in women over 10 yrs. Repeated, increased and may be due to either</td>
</tr>
<tr>
<td>1996</td>
<td>Belgrade</td>
<td>44° N</td>
<td>41.5</td>
<td>Pekmezovic et al. (62)</td>
<td>Both genders. Rate increasing</td>
</tr>
<tr>
<td>1999</td>
<td>Greece</td>
<td>36°-42° N</td>
<td>38.9</td>
<td>Piperidou et al. (63)</td>
<td>Incidence 2.36 (0.66 in 1978). Repeated, increased, and may be due to better ascertainment</td>
</tr>
<tr>
<td>1994</td>
<td>Sicily</td>
<td>37° N</td>
<td>4.4</td>
<td>Salemi et al. (64)</td>
<td>CDMS. 20% increase since 1989 due to decrease of time from onset to diagnosis</td>
</tr>
<tr>
<td>1995</td>
<td>Sicily</td>
<td>37° N</td>
<td>58.5</td>
<td>Nicoletti et al. (66)</td>
<td>CDMS, CPMS. Incidence 2.3</td>
</tr>
<tr>
<td></td>
<td>Wexford, Eire</td>
<td>52° N</td>
<td>120.7</td>
<td>McGuigan et al. (67)</td>
<td>Repeated, increased, and better ascertainment and diagnosis</td>
</tr>
<tr>
<td></td>
<td>Donegal, North Ireland</td>
<td>55° N</td>
<td>184.6</td>
<td>McGuigan et al. (67)</td>
<td>Latitudinal variation</td>
</tr>
</tbody>
</table>

(Continued)
Table 1  Prevalence (or Incidence) Rates of Multiple Sclerosis Reported in Recent Years (*Continued*)

<table>
<thead>
<tr>
<th>Year</th>
<th>Place</th>
<th>Latitude (approx.)</th>
<th>Prevalence/10^5</th>
<th>References</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>Colombia</td>
<td>0–15° N</td>
<td>1.48–2.98</td>
<td>Sanchez et al. (68)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Central Italy</td>
<td>42° N</td>
<td>53</td>
<td>Totaro et al. (69)</td>
<td>CDMS, CPMS. Both genders</td>
</tr>
<tr>
<td></td>
<td>Mexico</td>
<td>18–31° N</td>
<td></td>
<td>Velazquez-Quintana et al. (70)</td>
<td>No native Mexican patients</td>
</tr>
<tr>
<td>2002</td>
<td>Aragon, Spain*</td>
<td>39° N</td>
<td>75</td>
<td>Modrego and Pina (71)</td>
<td>Double 1994 rate. Incidence 4.6 now (NS). Repeated, higher, and due to improvement on case ascertainment</td>
</tr>
<tr>
<td>1993</td>
<td>South and West Finland*</td>
<td>60–63° N</td>
<td>111–202</td>
<td>Sumelahti et al. (72)</td>
<td>1.7×1983 rates only in some areas. Repeated and higher rates</td>
</tr>
<tr>
<td>1996</td>
<td>North Ireland*</td>
<td>55° N</td>
<td>190.7</td>
<td>McDonnell and Hawkins (73)</td>
<td>Repeated and same rates</td>
</tr>
<tr>
<td>2001</td>
<td>Newfoundland, Canada</td>
<td>48° N</td>
<td></td>
<td>Sloka et al. (12)</td>
<td></td>
</tr>
</tbody>
</table>

*The study has been revised from a previous one.

*Abbreviation: CDMS, clinically definite multiple sclerosis.*
(75). The incidence of MS among the U.S. National Health Survey participants (181 definite/probable patients) also increased significantly with latitude \( (P = 0.03, \text{trend}) \), but there was an attenuation of the north–south gradient over time (76).

Such variation of MS prevalence rates with latitude strongly suggests an environmental contribution to the natural history of the disease (77), the discrepancies between prevalences of regions at similar latitudes [e.g., in Finland (78) and in Sicily and Malta (79,80)] notwithstanding. Large variations in prevalence among geographically close regions suggest that locally specific etiologies (either environmental or genetic) contribute to disease pathogenesis. A plot of prevalence versus latitude in recently published prevalence studies (Fig. 3) displays their general correlation, though against this interpretation is the similarity in prevalence rates of immigrant Jews from Europe/America and native-born Jews of European/American origin (81).

**Stability of Incidence Rates**

Over the past several years regional prevalence figures have been updated to monitor disease trends. Justification for updating prevalences stems from changing disease criteria (82,83), better general awareness of disease, subsequent earlier referral with consequent shorter time from first symptoms to diagnosis, better availability of diagnostic equipment, and sometimes better access to a neurologists (84,85). In Table 1, we indicate the studies that have been revised. All incidence studies have shown either stability in rates or an increase, and most studies of prevalence show that this is increasing (74). In Newfoundland, Canada, a study in 1983 using clinical records, as well as those of the government healthcare plan, yielded a prevalence rate

![Figure 3](image-url)
for the island of 55/10^5 (86), but when this study was repeated using the same methodology 20 years later, the prevalence rate had risen to 94.4/10^5 and the previous regional disparities had equilibrated (87).

Most authors have concluded that the increase is due to better case ascertainment through better disease awareness, better access to diagnostic equipment and diagnosticians, a changing population ethnicity, longer life expectancy, and earlier age at diagnosis (72,88). However, a few (Table 1) have hypothesized that there is a real increase in their regional incidence. As these studies are the minority, one may speculate that such increases only reflect a global statistical variation. Since all studies show an increase in incidence, however, the general overall increase requires consideration, but only the repetition of studies over time with similar ascertainment methods and environments can provide an accurate estimate of disease trends. If there is a global increase in disease incidence, a careful analysis of the environmental and genetic factors may point toward the underlying etiology.

**Point Source Outbreaks and Clustering**

The clustering of disease is a compass to both genetic and environmental contributions to etiology (see previous section on regional variations for genetic contributions) and it is sometimes possible to separate the contributions of each. In the mid-1990s, several cases of MS were reported in a small, north-central Illinois community, once contaminated by heavy-metal exposure from a zinc smelter. Nine new cases of clinically definite MS occurred among local residents between 1971 and 1990, representing a statistically significant excess of new MS cases over the numbers expected (89). A survey in Finland also supported previous findings of an uneven geographic distribution of MS with an incidence of 8.7/10^5 in a western and 5.1/10^5 in a southern region. A rate of 11/10^5 in one domain was over twice as great as that found in a neighboring one (78). Increasing incidences for men, decreases for both sexes, and stable incidence rates were all reported in adjacent areas—a marked disparity. The incidence trends could not be explained by differences in case ascertainment and suggested to the authors that environmental factors had modulated the incidence of MS over the 15-year study period.

**Migration Studies**

Migrant studies indicate an environmental contribution to natural history depending on the specific observations of the study. If migrants keep their risk of disease regardless of their destination (e.g., cystic fibrosis), a genetic cause is presumed, but if their risk is modified (e.g., malaria), an environmental cause is considered. In MS, the risk modification is complex. Multiple migrant studies in MS (75,90) suggest that people who migrate before adolescence acquire the incidence rates of the region to which they have migrated. In contrast, people who migrate to a region after adolescence retain the incidence rate of the region in which they grew up (91). However, the children of those who moved from low- to high-risk areas have shown greater susceptibility than their parents, again supporting the operation of an environmental factor. This compelling evidence is consistent for migration from areas of high-risk to areas of low-risk (75), suggesting that part of the disease process depends on geographical location, possibly involving an environmental pathogenetic principle. However, there is also evidence that migration from areas of low risk to areas of high risk is not associated with a substantial change in risk (91). This diminishes
the strength of conclusions that can be drawn from all migrant studies but does permit the observation that both geography and age play some as yet undetermined role in the natural history of the disease.

Studies of the age at which people migrate suggest that a general age range might be important in the natural history of the disease, in terms of susceptibility to an environmental pathogen. Many studies on age-at-migration suggest that either a general age range (75,90) or a “critical age” at migration alter the risk of disease. This critical age tends to be close to 15 (thus, populations migrating before the age of 15 from high- to low-risk regions acquire a lower risk of susceptibility). The implication of these studies is that the risk of acquiring MS may be largely determined by the age of 15 years, but they were based on very small population sizes (91). In studies from Australia (92) and the U.S.A. (93), a relation between the age of migration and the change in risk of acquiring MS has been suggested, and it has been hypothesized that the critical age is not 15 but exists sometime within the latter part of the first two decades of life (75,91) and that susceptibility may extend from age 11 to 45 years (94).

We recently examined the critical age of susceptibility in Newfoundland, (220) and found a linear relationship between the age of menarche and the age at which the first symptoms occurred. This suggests that the initiation of disease may be related to the changes occurring in the body during puberty. The biologic plausibility of this interpretation is corroborated by observations made by others; in terms of hormonal effects on MS, the mean relapse rate decreases during all three trimesters of pregnancy (95,96) and tends to increase up to three months post partum (96). The premenstrual period also triggers exacerbations in a subgroup of females with MS (97). However, since neither the use of oral contraceptives nor parity are significantly associated with the risk of MS (98,99), other factors must also be involved in disease initiation.

### Latency Period

Latency periods (the time from exposure to clinical presentation of the disease) based on a hypothesized age at exposure have been estimated and ranges of nine years (100), 9 to 12 years (101), and 8 to 14 years (100), have been reported. These studies, combined with those on migration, suggest that MS is ordinarily acquired in early adolescence with a lengthy latency before symptom onset (94).

### INTERCURRENT FACTORS WITH POSSIBLE ASSOCIATION

Although the prevailing wisdom is that MS is an immune-mediated condition (102), it fulfills few of the criteria of an autoimmune disease (103). Rose and Bona (104) stated that “... with new knowledge gained from molecular biology and hybridoma technology, as well as the original Witebsky postulates, ... [evidence that] a human disease is autoimmune in origin includes direct evidence from transfer of pathogenic antibody or pathogenic T-cells; indirect evidence based on reproduction of the autoimmune disease in experimental animals; and circumstantial evidence from clinical clues.” But MS certainly cannot fulfill Koch’s postulates (direct evidence) due to the ethical problems of the necessary experiment, and satisfies their criteria only by the indirect evidence of experimental autoimmune encephalomyelitis (EAE). The problem with this is that there are important pathological as well as clinical differences between EAE and MS, as remarked by Chaudhuri and Behan (103), who have
argued persuasively that because the pathologies differ the presence of sensitized T-cells is nonspecific and there are no disease-specific immune markers for MS; this is in fact a metabolic neurodegenerative disorder in which infectious agents(s) could be involved either in direct damage to the white matter or in inducing inflammatory responses that secondarily affect the brain (105).

The evidence that MS is an autoimmune disease is indirect and based upon animal models (104). Different mechanisms suggested for disease initiation include epitope spreading, thymic dysregulation, and molecular mimicry, the latter based upon the hypothesis that microbial pathogens resemble human proteins and that exposure to these similar microbial epitopes may trigger an “auto” immune response against the host. This may be due to a direct homology between epitopes, bystander activation of host sensitive immune cells, or molecular mimicry. Therefore, infectious agents or other antigen-carrying vectors (diet, toxins, etc.) may be responsible for the activation of the autoimmune cascade. On the other hand, there are significant data that suggest that infectious agents(s) could be involved in direct damage to the white matter (105).

Many factors, whether geographical or event-based, suggest that environment plays a role in triggering MS and we will review new evidence on various environmental influences in initiating the disease (Fig. 4).

**Diet**

Prior ecologic correlations suggesting that a higher intake of saturated fats and a lower intake of polyunsaturated fats might increase the risk of MS have been contradicted by the results of Zhang (106) who found no relationship between intakes of total fat or major specific types of fat and the risk of MS.

**Toxins**

Sievers (107) examined all the reports that vaccinations caused or exacerbated MS, and determined that such studies indicate that vaccinations neither increase the risk of symptom exacerbation in patients with MS nor constitute a causative agent of the disease. The risk from aniline dyes or other substances used in the leather industry was found to be increased (108), but the role of organic solvents remains undetermined (109).

**Ultraviolet Radiation and Vitamin D**

The geographical variation in prevalence rates has prompted speculation that climate may play a role in disease initiation and susceptibility, so ultraviolet radiation (UVR)
has been studied closely, especially since it may have an immune-suppressive effect. An inverse association between MS prevalence and UVR has been reported from Australia (110). MS prevalence data for six Australian regions were compared with UVR levels of the largest city in each region. A close association was found between the theoretical MS prevalence predicted from UVR levels, and the actual prevalence and mortality from MS was negatively associated with both residential and occupational exposure to sunlight. Regional variation in MS prevalence, predicted by regional UVR levels, is consistent with the hypothesis that UVR exposure may reduce the risk of MS, possibly via T-lymphocyte-mediated immunosuppression (111,112).

Ponsonby (113) reviewed the epidemiological evidence that UVR plays a protective role in MS and other autoimmune diseases, noting that a gradient of increasing prevalence with increasing latitude has been observed for both MS and Type 1 diabetes mellitus in Europe and North America, with seasonal influences on disease incidence and clinical course. The authors considered that there may be a beneficial immunomodulatory role for UVR, but the data were inconclusive, although ultraviolet B can specifically attenuate these processes in part through an UVR-induced increase in serum vitamin D levels (113,114). The potential protective effect of vitamin D on the risk of MS was further examined in both of the U.S. Nurses’ Health Studies (NHS). The pooled age-adjusted relative risk (RR) comparing women in the highest quintile of total vitamin D intake at baseline with those in the lowest was 0.67 and the intake of vitamin D from supplements was also inversely associated with risk of MS. The relative risk comparing women with an intake of 400 IU/day or more with women with no supplemental vitamin D intake was 0.59 (115). The relevance of vitamin D receptor gene (VDRG) polymorphism in the pathogenesis of MS was investigated in 77 conventional MS patients and 95 healthy controls (116). The AA genotype and the [A] allele in the profiles were significantly more prevalent in MS patients than in controls ($P = 0.007$ and $P = 0.0321$, respectively), suggesting that VDRG polymorphism may be associated with susceptibility to MS. These data are excitingly suggestive; however, the precise method whereby vitamin D exerts such a protective effect remains undetermined.

**Stress**

Although some patients with MS report that stress can trigger disease exacerbations, a study of coping behaviors in 36 patients (mean age 44.4 years) with relapsing forms of MS indicated that coping strategies (including distraction, instrumental, palliative or emotional preoccupation) moderate somewhat only the relationship between stress and MS disease activity (117). Using logistic regression, Ackerman (118) showed that exacerbations are more likely during at-risk periods following major life events but are relatively independent of the threat level and type of stressor. Disability levels, medication usage, cardiovascular reactivity, baseline heart rate, and life event density explained 30% of the variance in the proportion of weeks “ill.”

While anecdotal reports and small (usually retrospective clinical or MRI) studies have shown that physical neuro-trauma and psychological or other stresses may precede the onset of MS or may influence its course, no clear causative relationship (at least between physical trauma and MS) has yet been proven. Nevertheless, the nervous and immune system interactions both through the hypothalamic-pituitary-adrenal axis and by autonomic pathways are putative mechanisms underlying any correlation that may eventually be proved (119–122).
Seasonal Variation

Seasonal variation in MS exacerbations have been noted (123); in Japan, attacks are most common in the warmest and coldest months of the year. The heat of summer in warmer, low latitude areas may be a risk factor for MS attacks. Seasonally changing concentrations of air pollutants predispose individuals to transmissible infections, induce systemic immune responses, and enhance existing peripheral inflammation, so may enhance the seasonal changes in MS relapse rates by increasing susceptibility to transmissible triggers (124).

Antecedent Infections

There is good evidence that one or more infectious agents may be responsible for the induction of MS (105) as suggested by the different geographic gradients in frequency among Europoids, changes in prevalence due to migration and the effect of age at migration, the suggestion of epidemics and clusters of cases in some small communities, and anecdotal reports. The infectious hypothesis is also supported by the different temporal patterns of the disease in different geographic areas and by the fact that the presence of oligoclonal bands in the cerebrospinal fluid (CSF) may indicate the presence of an infecting agent (125), though how such an external agent triggers induction or worsening of MS is unknown. As Gilden (125) suggests, infection may induce host immune-responsiveness in a damaging way. The response of the host or the infective event itself could lead to the production of an agent that induces relapse. Supporting this is the fact that stress has been shown to be capable of reactivating viruses latent in the central nervous system (CNS) or in mononuclear cells (125).

From another angle, the original observations of Leibowitz et al. (126) suggesting that the risk of MS is increased in subjects who spent their early years in households characterized by a high level of sanitation have been superbly re-examined by Bach (127), who concluded that some childhood infections actually seem to protect against immune-mediated diseases such as asthma, Crohn disease, and type 1 diabetes as well as MS, through bystander suppression, antigenic competition, or another (still undefined) mechanism.

There is, however, no evidence that any single agent is responsible either for induction or protection, and analytical studies testing the association between MS and various previous infections have not allowed definitive conclusions to be drawn (128). The following sections summarize some recent studies on the relation between antecedent infections and the induction of MS.

Paramyxoviruses

In previous editions of this book, the possible roles of paramyxoviruses such as measles and canine distemper virus were reviewed. A suggestive association was detected by inference, but proof of causality was considered lacking. One recent observation showed that MS patients experienced several childhood infectious diseases (varicella, rubella, mumps) at higher ages than did normal controls (129), but no further data have appeared on this subject and interpretation is impossible.

Influenza Virus

In a Polish study, Kazmierski (130) found a positive correlation between the incidences of influenza and of MS, both in the same year and five years later, but not
between the incidence of MS and other environmental factors, and suggested that influenza infection could precipitate MS onset.

**Epstein–Barr Virus**

Wagner (131) found a significant (100%) Epstein–Barr virus (EBV)-seropositivity and a significant lack of primary EBV infections among 107 patients with MS in comparison with age- and gender-matched healthy controls in north Germany, indicating that all of these MS patients had been infected with EBV before the development of MS. Although there were no differences in reactivities of EBV-specific anti–early antigen immunoglobulin G (-IgG), -IgM, and -IgA antibodies between each group, MS patients had significant lower anti–Epstein–Barr nuclear antigen 1-IgG antibody titers. This is a possible serological sign of defective control of the typically persistent latent EBV carrier state. Numerous other case–control and similar studies [systematically reviewed by Marrie (67)] are in disagreement about any relationship.

**Chlamydia pneumoniae**

Munger (132) examined the association between *Chlamydia pneumoniae* (Cpn) infection and MS in the two U.S. NHS. Among 32,826 women in the NHS and 29,722 women in the NHS II, 141 incident cases of definite or probable MS were documented. Cpn immunoglobulin G seropositivity was positively associated with risk of MS. This association did not change after adjusting for age at blood collection, ancestry, latitude of residence at birth, and smoking. Seropositivity for Cpn was moderately associated with risk of relapsing–remitting MS and strongly associated with the risk of progressive MS. These results support a positive association between Cpn infection and progressive MS. However, in a follow-up prospective study (133), the authors reported that neither seropositivity nor serum antibody levels suggested any association between Chlamydia infection and MS. In another study, Cpn-specific IgG titers were significantly higher in the CSF of MS than in controls, but these elevated titers did not significantly correlate with disease duration, disease course, clinical or MRI disease activity, and disability or presence of oligoclonal IgG (134). Overall, the association between MS and Cpn infection is judged to be weak (109).

**Herpesvirus 6 and 7**

The role of HHV-6 in MS is controversial and more extensive understanding of its neurotropism and association with disease is required (135). HHV-6 virus has been detected in MS plaques in the brain, and patients with MS have been shown to have an aberrant immune response to HHV-6 (136,137). A systematic review of 28 studies using 12 different experimental techniques (138) showed that four of these techniques did provide evidence for an association between HHV-6 and MS, but none showed a causal relationship. Tomsone (139) reported that the prevalence of HHV-6 was significantly higher in patients with MS than in those with nondemyelinating diseases of the CNS, with demyelinating diseases of the peripheral nervous systems, or in blood donors. HHV-6 viremia was found only in patients with MS, especially in the active phase of the disease. However, active HHV-6 infection could not be demonstrated in patients suffering from active clinically definite MS in Kuwait (140). Given the clinical implications of the presence of antibodies against HHV-6 in MS patients, viral reactivation cannot be excluded as an environmental factor (141).
Two forms of HHV-6 exist; the B variant accounts for human disease but the A variant has not hitherto been regarded as pathogenetic. HHV is neurotropic, acquired almost universally early in life, can be reactivated by infections or other physiological stresses, according to some reports induces IgM antibody responses in MS subjects when compared with controls, and is detectable within CNS cells in MS patients but not controls (though not all of these claims have been replicated). However, there is recent evidence that in patients with active relapsing–remitting MS (RRMS) there is a heavy viral load of HHV-6A (only) (142), raising the possibility that this is indeed an external pathogen of real relevance in MS precipitation. Only further studies that confirm these results will decide the issue.

A significantly higher frequency of HHV-7 reactivation in patients with peripheral nervous system demyelinating diseases has also suggested its association with demyelinating processes (140).

**Herpesvirus 1**

There is some evidence that HSV-1 may be implicated in the etiopathogenesis of MS. In one study (143), HSV-1 mRNA and DNA were found in a significant number of acute MS patients but not in the control group. The data are insufficient to allow further comment.

**Varicella Zoster Virus**

Although a recent survey showed that varicella zoster virus (VZV) infection occurred at an earlier age in an MS cohort than in controls (144), another review of the epidemiological evidence for the etiological role of VZV concluded that the five studies with the best methodology failed to show an increased risk of MS associated with varicella or zoster infections and that there was insufficient evidence for a role of VZV in the development of MS (145).

**Hepatitis B Vaccination**

Touze et al. (146) investigated the relationship between hepatitis B (HB) vaccination and a first CNS demyelinating event in adults and showed that adjusted odds ratios for the first CNS demyelinating event within two months following an injection of HB vaccine were 1.8 (CI 0.7–4.6) in the whole group. In cases with clinically definite multiple sclerosis (CDMS) or clinically probable multiple sclerosis (CPMS) only, the odds ratios were 2.0 (0.8–5.4) and 1.6 (0.4–5.6), respectively, thus ruling out any strong association between HB vaccine exposure and a subsequent demyelinating event.

Sadovnick (147) investigated MS in adolescents in British Columbia before and after an HB vaccination program was begun, finding no evidence of a link between HB vaccination and MS or other demyelinating disease. Monteyne (148) agreed that no scientific data supported a causal link between vaccination and MS and that the most plausible explanation for any observed temporal association between vaccination and MS is coincidence.

Such a conclusion is in line with that of numerous other studies, but using data from a British general practice database, Hernan et al. (149) detected a more than three-fold increase in MS incidence after three years following immunization. As pointed out by Naismith and Cross (150), the significance of this finding is made problematic by the fact that over 90% of the MS subjects in the database had never received the vaccine; by the possible selection of subjects for vaccination in the first place; by the winnowing process that led to only 11 of 713 original MS subjects being
used to come to this conclusion; and by the absence of reports of MS following actual HB infection. The question has thus been re-opened, but the balance of evidence seems to be against any causal association.

Other
Exacerbations of MS in the context of a systemic infection lead to a more sustained damage than occur with other triggers, but there is no indication that it is due to enhanced opening of the blood–brain barrier (151).

MS-associated retrovirus (MSRV) is a component of the human endogenous retrovirus (HERV)-W family, with gliotoxic and superantigenic properties. In one study, MSRV was detected rarely in healthy blood donors, in most patients with inflammatory neurologic diseases, and in all MS patients. This agent may be a marker for inflammatory neurologic disease (152).

Respiratory tract infections may precipitate disease onset (151,153,154) and exacerbations of MS have been shown to be associated with significantly higher plasma levels of intracellular adhesion molecule 1, an inflammatory marker (151). Seven of nine upper respiratory tract infections URIs, due to picornaviruses, were associated with a subsequent MS attack during the at-risk period (155).

As summarized by Stuve et al. (156) no one candidate pathogen has been accepted as the causal agent of MS, but the supposition that neurotropic agents could disrupt the blood–brain barrier, allowing the release of CNS autoantigens into the blood compartment and leading to the creation of lymphocytes sensitized to myelin or axons is plausible and makes fewer assumptions than other current theories. Stuve et al. also discuss the concepts of molecular mimicry and the activation of CD4+ T-cells by infectious agents as alternative pathogenetic models.

Associations with Other Diseases
MS has been associated with other diseases, both directly and inversely. It has been stated that individuals with MS have a genetic predisposition to autoimmunity in general (157). Epidemiological studies of the co-occurrence of autoimmune diseases has aided the understanding of MS (158), and their co-occurrence may be biologically plausible if they are shown to share common etiologic pathways in immune system dysregulation. Moreover, other disease states may act as direct triggers for the induction of MS, or may induce relevant protective or provocative factors within the host. Unfortunately, factors such as the presence of circulating T-cells activated against myelin and the presence of gamma globulin in the CSF are not specific for MS, and even the passive transfer of antibodies to self-antigens does not induce as MS-like disease (102). It is problematic as to whether the manifestations suggesting an autoimmune basis to MS are primary rather than secondary to the neuronal damage resulting from some other mechanism.

In Table 2 we list some diseases that have been claimed to correlate with MS. One set of disorders that does appear to share commonality with the hypothesized autoimmune etiopathogenesis of MS is discussed further below (Fig. 5).

Thyroid Disease
Some studies have demonstrated a significant co-occurrence of both Graves disease and Hashimoto thyroiditis with MS (175). One hypothetical explanation is that MS is a disease characterized by activated T-cells that give off a milieu of cytokines, notably IFN-γ. IFN-γ has been hypothesized to induce the autoimmune process
<table>
<thead>
<tr>
<th>Disorder</th>
<th>Conclusion</th>
<th>References</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>Inverse correlation</td>
<td>Tremlett et al. (159,228)</td>
<td>Retrospective matched case–control study</td>
</tr>
<tr>
<td>Eczema, dermatitis, rheumatoid arthritis, thyroid disorders, inflammatory bowel disease, type 1 diabetes</td>
<td>No association detected</td>
<td>Tremlett et al. (159)</td>
<td>But see comment on thyroid disease below</td>
</tr>
<tr>
<td>Prior tonsillectomy</td>
<td>No association</td>
<td>Broadley et al. (160)</td>
<td></td>
</tr>
<tr>
<td>Childhood infection with measles, rubella, mumps, varicella, pertussis, or scarlet fever</td>
<td>No association</td>
<td>Bager et al. (219)</td>
<td>From the comprehensive Danish database</td>
</tr>
<tr>
<td>Hyperandrogenism</td>
<td>Significantly more common in MS patients than in controls</td>
<td>Falaschi et al. (162)</td>
<td></td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>Increased expression in MS subjects</td>
<td>Kimura et al. (163)</td>
<td>Small numbers</td>
</tr>
<tr>
<td>Type I diabetes</td>
<td>Prevalence in people with MS 3× that in their healthy siblings ((P = 0.001)) and 5× rate in general population ((P &lt; 0.0001))</td>
<td>Marrosu et al. (164)</td>
<td></td>
</tr>
<tr>
<td>Autoimmune diseases ((\text{OR} = 92.2; 95% \text{ CI}, 12.1–700.2))</td>
<td>(\text{OR} = 3.8; 95% \text{ CI}, 2.0–7.1)</td>
<td>Zorzon et al. (165)</td>
<td>140 females, 131 controls only studied</td>
</tr>
<tr>
<td>Migraine</td>
<td>(\text{OR} = 8.7; 95% \text{ CI}, 1.0–75.4)</td>
<td>Zorzon et al. (165)</td>
<td>140 females, 131 controls only studied</td>
</tr>
<tr>
<td>Vaccination against measles</td>
<td>(\text{OR} = 92.2; 95% \text{ CI}, 12.1–700.2)</td>
<td>Zorzon et al. (165)</td>
<td>140 females, 131 controls only studied</td>
</tr>
<tr>
<td>Condition</td>
<td>Effect</td>
<td>Reference</td>
<td>Note</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
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<tr>
<td>HB vaccination</td>
<td>No effect</td>
<td>Tosti et al. (166)</td>
<td>No need to modify vaccination regime</td>
</tr>
<tr>
<td>Vaccination</td>
<td>No effect</td>
<td>Sievers (107) Offit and Hackett (167)</td>
<td>See text</td>
</tr>
<tr>
<td>Thyroid disorders</td>
<td>3× more common</td>
<td>Karni et al. (81)</td>
<td>Females only studied</td>
</tr>
<tr>
<td>Autoimmune thyroid disease (Graves’ disease)</td>
<td>Excess risk in first-degree relatives of probands with MS</td>
<td>Broadley et al. (168)</td>
<td></td>
</tr>
<tr>
<td>Autoimmune thyroid disease</td>
<td>Higher prevalence of ATT in male MS patients (9.4%) than in controls</td>
<td>Niederweiser et al. (169)</td>
<td>No significant differences in females, however</td>
</tr>
<tr>
<td>Presence of ANA and antithyroid autoantibodies (ATAbs)</td>
<td>Frequency of ATAbs in opticospinal MS higher than that in non-OSMS</td>
<td>Sakuma et al. (170)</td>
<td></td>
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<tr>
<td></td>
<td>(P = 0.007), but that of ANA similar in each group</td>
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<tr>
<td>Sjorgen syndrome (SS)</td>
<td>3–16× more common in subjects diagnosed with MS</td>
<td>De Seze et al. (171)</td>
<td>SS can mimic PPMS</td>
</tr>
<tr>
<td>Young-adult-onset Hodgkin lymphoma</td>
<td>Familial clustering noted</td>
<td>Hjalgrim et al. (172)</td>
<td></td>
</tr>
<tr>
<td>Psoriasis</td>
<td>Subgroup of patients with a first- or second-degree relative with psoriasis had early MS onset</td>
<td>Annunziata et al. (173)</td>
<td></td>
</tr>
<tr>
<td>Acute poliomyelitis</td>
<td>19 cases of MS found among 5652 polio patients when compared with 11.0 expected [SIR = 1.73 (1.04–2.74)]</td>
<td>Nielsen et al. (174)</td>
<td>Danish study; 149,364 years of follow-up, but small numbers of events</td>
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</table>

*Abbreviations: MS, multiple sclerosis; HB, hepatitis B; PPMS, primary progressive multiple sclerosis.*
observed in Hashimoto disease, so the increased availability of activated T-cells in MS may cause an increased frequency of Hashimoto disease in MS patients. MS, Hashimoto disease, and Graves disease may also share the dysregulation of apoptosis during chronic inflammatory states through the induction of Fas expression on normal cells (220). Chronic inflammatory states may cause active T-cells to produce cytokines that may induce Fas expression in organs distal to the original inflammatory site.

Co-occurrence of MS with other defined autoimmune diseases has been suggested but not proven (Table 2) and no autoantigen specific to MS has been identified (156).

NATURAL HISTORY AND CLINICAL VARIABILITY

Epidemiological study must also encompass the natural history and the phenotypic variations of diseases to ensure that the entities studied are indeed unique and that the emerging data can be applied specifically to each.

Prognosis

The short-term prognosis in early RRMS is worsened by factors such as later age of onset; multiple (especially pyramidal or cerebellar) onset symptoms; positive MRI findings at onset; a short interval between the first and second attacks; the attack frequency in the first two years; a progressive disease course; incomplete recovery from the initial attack; and higher baseline expanded disability status scale (EDSS) scores—the more of these factors, the worse the prognosis (175–179). However, the total number of relapses in the first two years of the disease has no prognostic value. It was reported that 58% of patients with a diagnosis of probable MS, who have positive MRI findings, will progress rapidly to clinically definite MS (178). Later age, the number of neurological functional systems involved, sphincter, or motor–sensory symptoms and the presence of sequelae after onset are all valid predictors of the time to progression (180).

In the longer term, data on 248 patients in a prevalence study in Northern Ireland (181) indicated that 29% were fully independent in all basic activities of daily living (ADLs) of bathing, dressing, grooming, and feeding; 23% were unable to climb a flight of stairs; and 42% acknowledged problems with sexual function. Sixty-one (25%) were working essentially full-time, but 53 (21%) had no external financial support. Twelve (5%) were institutionalized and 86 (35%) required assistance with activities of daily living (ADLs) for at least 1 hr/day. Eighty-one (33%) were unable
to drive a car or use public transport. Forty-two (17%) were accessing community services for at least 1 hr/day on average.

A prospective study (182) of 54 subjects (36 female) with CDMS and with disease onset at 15 years or earlier gave a female-to-male ratio of 4.7 in subjects with age ≥12 years, suggesting a role of hormonal changes in triggering MS onset. Over a mean follow-up duration of 10.9 ± 5.6 years, it was found that the onset was monosymptomatic in 57%. The functional systems more frequently involved at onset were the pyramidal and brainstem (28% each); and the course was relapsing–remitting in 72% and relapsing-progressive in 28%. Disability after eight years was highly predicted by disability in the first year (\( P = 0.008 \)) but this outcome was not influenced by the characteristics of symptoms at onset, age, or gender.

In a five-year study of 83 patients with clinical onset of MS aged < 16 years and of 710 with onset between 16 and 65 years adult-onset multiple sclerosis (AOMS). Simone et al. (183) showed that the EDSS evaluated at the last clinical examination was lower in those with earlier onset multiple sclerosis (EOMS), despite a longer disease duration. Median times to reach EDSS score of 4 and secondary progression were longer in EOMS than in AOMS, but the age at both endpoints was significantly lower in EOMS. In both EOMS and AOMS, irreversible disability was related to a secondary progressive course, sphincter involvement at onset, and an older age at onset. In adult onset cases, other unfavorable factors were pyramidal involvement at onset and a high relapse frequency in the first two years. The risk of developing secondary progressive MS (SPMS) was increased by a high number of relapses in early, and by a higher age at onset, and a short inter-attack interval in adult onset disease. Both these studies are in agreement with the findings of Hawkins and McDonnell (179) (see below).

Such findings are hardly surprising because MS is likely to be an axonopathy and the cumulative damage to axons with minimal ability for repair must lead to earlier and faster clinical deterioration, although the gender difference remains unexplained.

**Age**

Clinical disability in MS is influenced by the patient’s age \( (P < 0.01) \) rather than by the age at onset (184). EDSS scores increase in parallel with age and duration of disease \( (P = 0.007) \) (187). Median times to reach EDSS scores of 4 and 6 are significantly longer among patients aged 20 to 35 years compared with those aged 36 to 50 years and 51 to 65 years \( (P < 0.0001) \). Significant associations were observed between mean EDSS scores and age at disease onset, current age, and the interaction of age at disease onset and current age \( (P < 0.001) \). An age-adjusted progression index may be a more relevant criterion for defining differences between MS groups.

Moris et al. (185) evaluated 55 MS patients [46 CDMS, 9 CPMS; 33 RRMS, 11 SPMS, and 11 primary progressive MS (PPMS)] longitudinally from onset of the disease over a mean duration of seven years. The mean age of onset was 31.1 years, pyramidal weakness and sensory symptoms being the most common initial problems. The median times to reach EDSS-3 and EDSS-6 from onset were 4.5 and 7.5 years, respectively. The average time from onset of MS to secondary-progressive was 6.1 years. There were no significant differences between treatment and nontreatment patients.

In a retrospective review of the clinical protocols of 17 patients with young onset MS (first symptoms before 21 years) (186), the mean age at onset was 16.9 ± 4.4 and median time to diagnosis was four weeks. The clinical course was relapsing–remitting in 76.5% and secondary progressive in 23.5%. The mean annual
exacerbation rate was 1.5 ± 0.9 and median time to second exacerbation was 12 months. The actual EDSS score was 2.6 ± 2 after a mean disease duration of 11.4 ± 8.0 years. The only statistically significant result was a correlation between the EDSS and the mean disease duration. Age at onset did not correlate with final neurological disability.

Among 640 patients with the first presentation of clinical symptoms of MS (Poser criteria and brain MRI), 30 (4.6%) were diagnosed as suffering from late-onset MS. In half of them the initial disease course was relapsing–remitting. Motor symptoms were the most common neurological presentation. Major depressive episode was diagnosed in 6 out of 30 patients (20%) in the two years prior to the diagnosis of MS. Late-MS onset may present as major depression, progression to disability is more rapid, and a primary progressive course is more prevalent (187).

Benign MS

Although this form of the disease is usually considered to occur in 10% of subjects, among 23 patients followed up for 10 or more years, 11 (48%) were so diagnosed (188). It seems that the longer the duration of stable MS to date and the less the initial disability, the more likely a patient is to remain stable without progression, especially for the patients who have “benign MS” by virtue of their EDSS scores of ≤2 for ≥10 years. These subjects have a >90% chance of remaining stable (189) and are most often relatively young females who experienced ON or sensory rather than motor symptoms at onset (179). However, further follow-up more than 10 years after the initial ascription of the “benign” status indicates that this is not necessarily permanent.

Optic Neuritis

Reviewing nearly 100 cases of isolated ON, Ghezzi et al. (190) found that about a third developed CDMS after a mean of 2.3 years. The risk was 13% after two years, 30% after four years, 37% after six years, and 42% after 8 and 10 years. These figures were not affected by gender, age, or season of onset. The 10-year risk of MS following an initial episode of acute ON is significantly higher in the presence of a single brain MRI lesion, but larger numbers of lesions do not appreciably increase that risk (191), and in those that progress to CDMS, disability remains relatively mild for at least the first 10 years (192). Most patients with a diagnosis of probable MS and positive brain MRI will progress rapidly to clinically definite MS (178).

In Pokroy’s study of 10 black South African patients with ON (193), only two had truly unilateral ON, the others had either bilaterally simultaneous or consecutive disease. After at least three months follow-up, only six eyes recovered visual acuity of 6/12 or better, and only three eyes recovered color vision of 10/13 or better. No patient had clinical MS on presentation, nor developed it on follow-up. The higher the prevalence of bilateral cases and optic disc swelling, the weaker the association with MS, and the extremely poor visual outcome distinguishes ON in black South Africans. In a large U.S. study (194) the five-year conversion rate from ON to neuromyelitis optica (NMO) was 12.5% and 14.4% to MS. Patients with a rapid succession of severe ON events were found to be more likely to develop a generalized demyelinating disease.

Predictors of a relapsing course after an episode of NMO were longer inter-attack interval between the first two clinical events [rate ratio (RR) = 2.16 per month
increase], older age at onset ($RR = 1.08/yr$ increase), female sex ($RR = 10.0$, female vs. male), and less severe motor impairment with the sentinel myelitis event ($RR = 0.48/severity$ scale point increase). A history of other autoimmune disease ($RR = 4.15$ for presence vs. absence), higher attack frequency during the first two years of disease ($RR = 1.21/attack$), and better motor recovery following the index myelitis event ($RR = 1.84/point$ increase) were associated with mortality due to relapsing NMO (195).

**Primary Progressive Multiple Sclerosis**

MS has been clinically classified by an international survey of MS experts (196) as relapsing–remitting (RRMS), secondary progressive (SPMS), and primary progressive (PPMS) disease. PPMS has been defined as that form showing disease progression from onset, though occasional plateaus and temporary minor improvements are allowed (196). Between 10% and 15% of MS, patients follow a primary progressive course with a distinct clinical and paraclinical phenotype (197). However, the male-to-female ratio is lower than RRMS (1:1.5–2) (198) and patients with PPMS are more likely to present with progressive myelopathy and at a later age (37 years for PPMS vs. 31 years for RRMS) (87,198). The rate of deterioration from disease onset is substantially more rapid than for RRMS, with a median time to disability status score (DSS) 6 and 8 of 8 and 18 years, respectively. Life expectancy, cause of mortality, and familial history profile are similar in PPMS and non-PPMS. The mean time to death is decreased when more neurological systems are involved at the onset of disease but age, gender, and neurological system involved at onset appear to have little influence on prognosis (199).

Even though their clinical courses are different, PPMS and RRMS may have similar HLA haplotype associations (200) but in comparison with RRMS, there are fewer lesions on MRI (201), higher in vitro migration, differences in immune cell products (202), and less inflammation on necropsy (201).

New evidence suggests that the spectrum of disease may also be delineated along pathophysiological boundaries, which may or may not correlate with the clinical/genetic boundaries suggested above (203). It has been suggested that one form of MS may be characterized by inflammation directed against myelin while another form is due to progressive axonal degeneration (204,205). Whether the final pathophysiological categorization of MS correlates with the clinical/genetic categorization of MS remains to be established.

**Psychiatric Features**

Suicidal intent (a potential harbinger of suicide) is common in MS and is strongly associated with major depression, alcohol abuse, and social isolation (206). Anxiety is a frequent accompaniment to depression in MS (207). A major depressive episode was diagnosed in 20% of cases in the two years prior to the diagnosis of (late-onset) MS, which may therefore be considered as able to present as major depression (187).

**CLUES TO ETIOLOGY**

Prevalence data imply that racial and ethnic differences are important in influencing the worldwide distribution of MS and that its geography must be interpreted
in terms of the probable discontinuous distribution of genetic susceptibility alleles, which can, however, be modified by environment (1). But the story does not end there, because variability in the populations studied, the use of different diagnostic criteria, and variable levels of ascertainment must all reduce our confidence in the meaningfulness of the data currently available. Nevertheless, it seems indisputable that MS is a degenerative disease of the CNS resulting from an externally derived attack, occurring only in a proportion of those people who are genetically susceptible. As this chapter has shown, complete understanding of the etiopathogenesis of the disease is still elusive. Many hypotheses regarding potential exogenous provokers of MS have been suggested, as discussed above and in Table 3, but almost all remain controversial. The defined but complex genetic interrelationships have defied interpretation and the conclusion can be drawn that a genetic predisposition appears to be a necessary (but not a sufficient) factor to confer susceptibility to MS. Further complicating the interpretation of the data, it is by no means certain that MS is a single disease entity (102); MS may represent a spectrum of disease.

The following genetic, epidemiological, and environmental clues have been repeatedly verified, but it is tantalizing that they are still insufficient to rationalize a theory of etiopathogenesis.

**Incidence and Prevalence Rates Have Increased.** Updated epidemiologic data in the context of new diagnostic criteria have more accurately characterized the spatial, if not the temporal, distribution of the disease. Our figures from Newfoundland show a near-doubling of the prevalence rate over 20 years, but we cannot incriminate any specific familial or infectious factors for this. Rather, the better availability of neurologists, heightened awareness of the disorder, and an improvement in available diagnostic techniques seem to be most likely responsible in this Canadian province, and probably elsewhere.

**There Is an Important Genetic Component.** This is witnessed by family history studies, demonstrating concordance rates for monozygotic twins that affirm a genetic influence in the disease; by documented racial susceptibilities; and by the variation of the proportion of disease subtypes between races (the opticospinal variant, NMO, occurs more frequently in east Asians than in other races). Racial variations in susceptibility can be explained using isolation of genomes in combination with distant founder effects, especially in races where extra-racial intermarriage has been rare. Although races with isolated genomes show differences in recessive and multifactorial diseases due to differences in allele frequencies when compared with other races, not all racial variations can be explained strictly by genetics—some may be attributed to culture and environment, as evidenced by the frequency of Kuru in certain Papua New Guinean populations.

An increased familial risk has been repeatedly demonstrated. It tends to decrease the further one, which is related from the proband, implying a strong genetic role. Thus, monozygotes have an increased risk over dizygotes, who have the same risk as other siblings, and have a higher risk than first-degree cousins. There is no difference between maternally versus paternally transmitted risk, although children of two affected parents have a 10 times risk over children with only one affected parent. Adoptees and spouses have the same risk as the local population, arguing against an isolated environmental risk in families with MS.

There is no evidence for any specific Mendelian inheritance pattern. The sib risk is not the 25% or 50% that one would expect with a pure recessive or dominant disease and the twin concordance rate is far from 100%. (Curiously, the risk of parents with affected children is higher than with recessive disease while the risk
The risk is always greater for females and female relatives, so neither is this X-linked inheritance. Moreover, full scale genomic searches in sib pairs have suggested that no specific region of the genome plays a major role in susceptibility. One might speculate as to whether there is a susceptibility factor on the X chromosome or a protective one on the Y.

### There Are Age and Gender Differences in Disease Presentation and Prognosis.

Females are at least twice as often affected as males; in women the disease presents

### Table 3  External Influences Examined for Their Effect in Producing Multiple Sclerosis

<table>
<thead>
<tr>
<th>External influence</th>
<th>Conclusion</th>
<th>References</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>School teaching</td>
<td>Excess mortality among schoolteachers from autoimmune diseases</td>
<td>Walsh and DeChello (208)</td>
<td>Presumed to be due to early occupational exposures</td>
</tr>
<tr>
<td>Ionizing radiation</td>
<td>Excess MS in subjects exposed to ionizing radiation</td>
<td>Axelson et al. (209)</td>
<td></td>
</tr>
<tr>
<td>Inhaled radon gas</td>
<td>Considered to be a risk factor</td>
<td>Bolviken et al. (210)</td>
<td></td>
</tr>
<tr>
<td>Smoking and certain infectious diseases</td>
<td>Causative effect</td>
<td>Ghadirian et al. (211) Hernan et al. (212)</td>
<td></td>
</tr>
<tr>
<td>Welding</td>
<td>No effect</td>
<td>Hakansson et al. (213)</td>
<td></td>
</tr>
<tr>
<td>Oral contraceptive use</td>
<td>No lasting protective effect</td>
<td>Hernan et al. (98)</td>
<td>Prospective study</td>
</tr>
<tr>
<td>Exposure to mercury</td>
<td>No effect</td>
<td>Casetta et al. (214)</td>
<td></td>
</tr>
<tr>
<td>Dental caries, mercury, and lead exposure</td>
<td>21% increase in MS with caries only</td>
<td>McGrother et al. (215)</td>
<td>Suggests geographical association</td>
</tr>
<tr>
<td>Inhaled particulate matter</td>
<td>Increases trigger MS relapses</td>
<td>Oikonen et al. (124)</td>
<td></td>
</tr>
<tr>
<td>Exposure to organic solvents</td>
<td>Double rate of MS disability pensions awarded to painters when compared with those not exposed</td>
<td>Riise et al. (216)</td>
<td>16 ys follow-up study from Norway</td>
</tr>
<tr>
<td>Environmental pollution</td>
<td>4× risk of MS relapses (4.143, ( P &lt; 0.001 )) when the concentration of inhalable particulate matter was at the highest quartile</td>
<td>Oikonen et al. (124)</td>
<td>SW Finnish study</td>
</tr>
<tr>
<td>Inhalable airborne particulate matter; ambient air pollutants</td>
<td></td>
<td>Spirin (217)</td>
<td></td>
</tr>
<tr>
<td>Organic solvents De-worming</td>
<td></td>
<td>Weatherby (161) Weinstock et al. (218)</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviation: MS, multiple sclerosis.*
at a relatively fixed time following menarche; the prognosis for disease development is worse in males and in those with older age of onset or with polysymptomatic signs (especially pyramidal and cerebellar); and it is better in younger females with monosymptomatic sensory or optic involvement. The operation of an undefined hormonal factor may account for the gender-related findings. Paradoxically, the younger the age of onset, the better the prognosis.

There Is Repetitive Evidence of Increasing Equatorofugal Prevalence Rates. Such data incriminate some factor related to the geographical environment. In this context it is noted that there is a fair correlation between sunlight and/or other sources of vitamin D and prevalence rates worldwide, but the mechanism of this remains elusive.

Clinically Defined Variants Exist. These variants include relapsing–remitting/secondary progressive MS (which are surely the same condition at different stages of development); a form that appears to be benign for years; and yet another that remains subclinical; a primary progressive type; the opticospinal variant; and acute lethal MS (the Marburg variant). Genetic predispositions are likely to be responsible for such phenotypic variability, but the nature of the inciting external agents provoking the specific clinical variants is unknown and the boundaries of immunopathological classification may not fully correlate with the classification of accepted clinical variants. In sum, the clinical variability of MS indicates that at least PPMS and that form heralded by ON have different natural histories and may represent separate etiopathologic entities.

Thyroid Disease Is Unusually Frequent in Populations of MS Patients. Whether this is evidence of a shared tendency to autoimmune disease or whether both the CNS and the thyroid are susceptible to attack by the same inciting agent remains undetermined.

Intercurrent Challenges to the Immune System Such As Vaccination, Infections, and Poor Air Quality Can Precipitate MS or Its Relapses. Recent evidence that an unusual form of HHV (type A) is detectable in the brains of MS patients, its viral load in the blood correlates with relapses of MS, and that this neurotropic virus is almost universally present and can be reactivated by stressful events (99) tie together these observations, but await confirmatory proof. Most vaccinations and infective agents have been shown not to correlate with disease initiation or course.

In the Northern Hemisphere There Is a Significant Excess of Births in May Compared with Those in November. Some external influence appears to operate upon the fetal environment during the northern hemispheric winter months.

There seem to be two questions that one can try to answer on the basis of the data discussed above.

Is MS a Polygenetic Disease with Reduced Penetrance?

Observations from twin, family, and racial studies leave little doubt that there is a genetic component to the etiology of MS (Fig. 1), the risk increasing with genetic similarity; but the mode of inheritance is neither purely recessive, nor dominant or X-linked. That MS is a genetic disease with multiple genes that contribute variably to both phenotype and susceptibility may be close to the truth, but it cannot be the whole truth, since 100% concordance between twins is not observed, thus ruling out any pure Mendelian mechanism.

MS might be a late-onset polygenetic disease with reduced penetrance, but one must search for the cause of the reduced penetrance in the context of the absence of a cost-effective screening method. In order to study penetrance in a family, a reliable
means of diagnosis is necessary to make conclusions about the penetrance rate. Usually this is a known gene. However, (new paraclinical contributions to diagnosis notwithstanding) MS is a clinical diagnosis and the nature of any responsible gene or set of genes remains unknown. Therefore, familial studies are biased in two ways: subclinical disease is not adequately accounted for, and verifiable genetic markers of disease susceptibility are unavailable.

Even though reduced penetrance is a possible contributing factor, it is difficult to quantify a penetrance rate because of this bias. A molecular/genetic basis for reduced penetrance can arise from variation in the action of gene transcription modulators which can be strictly genetic (e.g., trinucleotide repeats of varying length of local gene transcription modifiers) but might also include factors that are remotely influenced by action through cell and nuclear membrane receptors. The latter (receptor-influenced) factors suggest a mechanism for external influence, so that environmental factors may indeed influence disease initiation and induction.

In conjunction with observations based on both hormonal and gender influences, a further observation relevant to the effects of environment stands out. Hormones (including vitamin D) are derived from steroids, are lipid soluble molecules, and may act on nuclear membrane receptors. The separate discussions on menarche, vitamin D and related hormone studies above, and variation of MS with birth month, coupled with observed gender differences in both disease course and susceptibility suggest a likely role for hormones in either the induction of the disease or susceptibility to it.

**Is MS a Virally-Induced Autoimmune Disease?**

Not mutually exclusive to the polygenetic hypothesis presented above, a viral cause for MS (either as a primary mechanism of neuronal attack or secondary to an induced immune/inflammatory process) has had continued attention for years. While no specific virus nor other infectious agent nor any specific vaccine has been definitively incriminated as a causative factor in MS occurrence or deterioration, an association between infections or other stresses and the unmasking or deterioration of MS has been shown repeatedly. The range of putative causative agents is wide. For disease induction, it is perhaps necessary that repeated exposures to causative agents occur in a certain contextual environment. (Fig. 1). Molecular mimicry via shared epitopes from an unknown viral influence has been suggested as a mechanism for disease induction in purported autoimmune diseases (such as type I diabetes, Hashimoto disease, and MS). Although the classification of MS as an autoimmune disease remains controversial, immune dysregulation has been repeatedly observed and genetics may play a role in susceptibility to immune dysfunction. The simplest explanation might be that such clinical deteriorations represent a nonspecific reaction to an immune or hormonal challenge.

While this summarizing statement is hardly original, it seems that MS is the end result of a re-awakening by stressors of a latent neurotropic virus, or some other pathogen capable of causing axonal and (perhaps indirectly) oligodendroglial damage within the CNS. The precise mode of operation of such stressors still awaits discovery. This may be as far as the evidence takes us today, but although in the last five years we have moved forward only slowly in the hunt for the etiology of MS, the data reviewed here perhaps orient us more reliably to the pathways of future research that are most likely to be fruitful.
REFERENCES


Genetics: Susceptibility and Expressivity

Thomas Masterman and Jan Hillert
Division of Neurology, Department of Clinical Neuroscience, Karolinska Institutet, Karolinska University Hospital, Huddinge, Stockholm, Sweden

SUSCEPTIBILITY

Linkage

It has long been recognized that, despite living at geographical latitudes where multiple sclerosis (MS) is common, genetically isolated ethnic groups—including Gypsies in Hungary (1); Indians and Orientals in North America (2); Aborigines in Australia (3); and Maoris in New Zealand (4)—remain resistant to the disease. Systematic analysis of familial aggregation of MS—in particular, studies of twins (5–8), adoptees (9), and half-siblings (10)—has also confirmed Eichhorst’s description from the 1890s of MS as a “heritable” disorder (11). The degree to which a disease is heritable can be estimated by dividing the lifetime risk of siblings to affected individuals by the population prevalence of the disease, to yield the so-called \( k \) statistic. For MS, in high-risk populations, \( k \) is between 20 (0.02/0.001) and 40 (0.04/0.001)—a value similar to that seen in insulin-dependent diabetes mellitus (12). Data from twin studies—which show that the concordance rate of approximately 30% in monozygotic twins drops steeply to a rate below 5% for dizygotic twins—strongly indicate that susceptibility to MS is influenced by many genes in combination (13).

To date, studies conducted with the goal of identifying susceptibility-conferring genes in MS have for the most part taken the form of either linkage screens, in which the segregation of polymorphic microsatellite markers, located throughout the entire genome or at candidate loci, is analyzed in collections of multicase MS families, or association studies, in which genotype frequencies at polymorphic positions in or near selected genes are compared in sporadic MS cases and ethnically matched healthy controls. In 1996, the results of three large multi-stage genome-wide screens performed on datasets of affected relative pairs collected in the United Kingdom (14), the United States and France (15), and Canada (16) were published; results from a fourth Finnish screen (17) appeared the following year. Each screen uncovered multiple loci of potential involvement in MS, supporting genetic-epidemiological suggestions of polygenic inheritance. A number of loci—including the HLA region on chromosome 6p21—were positive for linkage in more than one study.

In 2001, a meta-analysis was performed on the raw genotyping data from the British, Franco-American, and Canadian screens (18). Eight chromosomal regions...
displayed nonparametric linkage (NPL) scores greater than 2.0: 17q11, 6p21, 5q11, 17q22, 16p13, 3p21, 12p13, and 6q tel (in descending order). For no region, however, did NPL scores reach levels indicative of genome-wide significance. The authors offered two alternative explanations for the “failure” of their meta-analysis: “The first is that genetic factors with substantial effects do not exist and susceptibility to the disease is more likely determined by many genes, each exerting a relatively modest effect, acting together. The other possibility is that genes with large effects do exist in some families, but because of the genetic complexity of MS, these genes cannot be defined in a heterogeneous outbred population.”

Since the publication of that meta-analysis, five additional genome-wide linkage screens have been performed on MS families from mainland Italy (19), Sardinia (20), the Nordic countries (21), Australia (22), and Turkey (23). In addition, as part of the Genetic Analysis of Multiple Sclerosis in Europeans (GAMES) project discussed below, a renewed meta-analysis was performed (24), which incorporated data from all nine genome screens (Table 1). Although the number of non-HLA regions exhibiting NPL scores greater than 2.0 had now been narrowed down from seven to four—11ptel, 16p13, 17q21, and 22q13—only one region exceeded the threshold for genome-wide statistical significance: HLA on 6p21.

Table 1  Regions Displaying Positive Linkage in Nine Genome-Wide Linkage Screens of Multicase Multiple Sclerosis Families and One Meta-Analysis of These Screens

<table>
<thead>
<tr>
<th>Chr</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr1</td>
<td>p35(C), p21(U), q11–24(N), q31(S), q42–44(l,A)</td>
</tr>
<tr>
<td>Chr2</td>
<td>p23(Am), p21(C), p13(A), q24–33(G,F,N), q36(l)</td>
</tr>
<tr>
<td>Chr3</td>
<td>p26(N), p25(C), p14(C), q21–24(Am,C,F,N,A), q26(C)</td>
</tr>
<tr>
<td>Chr4</td>
<td>p16(C), q12(F,N), q24(A), q26–28(C,A), q31–35(Am,A)</td>
</tr>
<tr>
<td>Chr5</td>
<td>ptel–14(C), p14–12(F), q11–13(U,A), q13–23(Am,C), q33(l)</td>
</tr>
<tr>
<td>Chr6</td>
<td>p25(I,N), p21(Am,F,N,M), q14(C), q21(N), q22(l), q26(A), q27(A)</td>
</tr>
<tr>
<td>Chr7</td>
<td>p21(C), p15(U), p14(C), q11(Am), q21–22(Am,C), q32–35(Am,A)</td>
</tr>
<tr>
<td>Chr8</td>
<td>p23–21(A)</td>
</tr>
<tr>
<td>Chr9</td>
<td>p24–22(Am), q21(A), q34(Am,N)</td>
</tr>
<tr>
<td>Chr10</td>
<td>p15(Am), p12–12(N), cen(l), q21–22(Am,F), q24(S), q26(C)</td>
</tr>
<tr>
<td>Chr11</td>
<td>ptel(M), p15(Am,S,N), q22(C), q25(F)</td>
</tr>
<tr>
<td>Chr12</td>
<td>p13(U), q21(N), q23(Am), q24(Am)</td>
</tr>
<tr>
<td>Chr13</td>
<td>q14–22(T), q31–32(A), q33–34(Am)</td>
</tr>
<tr>
<td>Chr14</td>
<td>q32(C)</td>
</tr>
<tr>
<td>Chr15</td>
<td>q21(C,l)</td>
</tr>
<tr>
<td>Chr16</td>
<td>p13(Am,N,A,M), p11(A), q12(C), q23–24(A)</td>
</tr>
<tr>
<td>Chr17</td>
<td>p13(A), q21(M), q22–24(U,F), q25(N)</td>
</tr>
<tr>
<td>Chr18</td>
<td>p11(Am,C,F,A), q21(C), q23(T)</td>
</tr>
<tr>
<td>Chr19</td>
<td>q13(Am,C,F)</td>
</tr>
<tr>
<td>Chr20</td>
<td>p12(A)</td>
</tr>
<tr>
<td>Chr21</td>
<td></td>
</tr>
<tr>
<td>Chr22</td>
<td>q12–13(U,N,M)</td>
</tr>
<tr>
<td>ChrX</td>
<td>p22(C,N), p21(C,A), p11(C,A), q23–28(A), q26(C)</td>
</tr>
</tbody>
</table>

Note: U, United Kingdom, (14), maximum LOD score (MLS) > 1.8; Am, United States and France, (15), positive in at least two-thirds of tests; C, Canada, (16), 56% sharing; F, Finland, (17), nonparametric linkage (NPL) > 1.0; I, Italy, (19), LOD > 0.7; S, Sardinia, (20), MLS > 1.8; N, Nordic countries, (21), LOD > 0.7; A, Australia, (22), MLS > 0.7; T, Turkey, (23), MLS > 1.8; M, meta-analysis, (24), NPL > 2.0.

Source: From Ref. 25.
HLA

The HLA complex spans about 4 Mb on the short arm of chromosome 6. It harbors dozens of genes, many of which encode proteins involved in the immune response, including the highly polymorphic polypeptide chains of the HLA class I and class II molecules. The chains encoded by the HLA-A, -B, and -C genes, located in the telomeric class I region, are expressed on the cell surface of virtually all nucleated cells; complexed with β2-microglobulin, they present peptides derived from cytosolic antigens, e.g., self antigens and the products of intracellular pathogens, to cytotoxic CD8+ T cells. The HLA-DR, -DQ, and -DP genes of the centromeric class II region encode the α and β chains of the heterodimeric cell-surface molecules that present endocytosed antigens, e.g., extracellular pathogens, to CD4+ “helper” T cells. The HLA class III region, located between the class I and class II regions, also contains a number of polymorphic genes encoding components of the immune system—such as complement factors and TNF-α and -β—but none encoding “classical” peptide-presenting HLA molecules.

In the early 1970s, Jersild et al. (26) first reported an association between MS and the HLA class I alleles, A3 and B7, and a year later, a stronger association to the class II specificity Dw2 (27). It became apparent that the former association was secondary to the latter, a result of the high degree of linkage disequilibrium (LD) in the HLA complex, whereby strings of alleles at adjacent loci escape separation by meiotic recombination and are inherited together as conserved haplotypes. The MS-associated HLA haplotype, whose boundaries have now been determined by genomic techniques, consists of alleles of four adjacent class II genes—DRB1*1501, DRB5*0101, DQA1*0102, and DQB1*0602. Although the haplotype is most common in Scandinavia, it appears to be increased, compared to frequencies in controls, in MS patients from all ethnic groups (28).

The extensive conservedness of this haplotype—the infrequency with which its component alleles occur unaccompanied by the others—makes it difficult to determine which part of haplotype is responsible for the susceptibility-conferring biological phenomena underlying the genetic association to MS. Recently, however, Oksenberg et al. (29) investigated a dataset of African American MS patients and controls—a population exhibiting greater haplotypic diversity than northern Europeans—and uncovered an association with HLA-DRB1*15, in the absence of DQB1*0602. This finding suggests that it is the DRB1 gene—or rather the DRβ chain it encodes—that plays a functional role in etiopathogenesis of MS. In an earlier study, however, Caballero et al. (30), comparing a group of Brazilian MS patients of African origin with a group of ethnically matched controls, observed in patients an increase in the frequencies of DQA1*0102 and DQB1*0602, in the absence of DRB1*1501, implicating the DQ molecule as the functional culprit.

Meanwhile, Ligers et al. (31) found evidence of linkage to the HLA-DRB1 locus in 58 DRB1*1501-negative Canadian MS families, suggesting the existence either of a hierarchy of predispositional and protective DRB1 alleles; or of a primary, non-DRB1 susceptibility locus in strong LD with the DRB1*1501 allele (25). Indeed, in a study of the Sardinian population, Marrosu et al. (32) demonstrated not only the presence of four independent MS susceptibility loci within the HLA complex—at the DRB1, DQB1, and DPB1 loci, as well as at a locus telomeric to the classical class I genes—but also the positive association of five DRB1-DQB1 haplotypes with MS. To complicate matters even further, carriage of the HLA class I allele A*0201 appears to decrease the risk of MS (33), while
studies by Barcellos et al. (34) and our own group (35) have demonstrated a dose
effect of the serologically defined risk specificity HLA-DR15 (Table 2).

Although the mechanism by which alleles of classical or nonclassical HLA
genes might predispose carriers to MS is still unknown, the following models have
been proposed (36):

1. **Determinant model.** Carriage of the MS-associated HLA genotype facili-
tates presentation of encephalitogenic peptides to CD4+ T cells.

2. **Thymic-selection model.** Deletion of encephalitogenic T cells in the
thymus is compromised by the presence of the MS-associated HLA
genotype.

3. **Molecular-mimicry model.** The MS-associated HLA genotype is associated
with presentation of bacterial or viral peptides with structural homology to
autoantigens of the central nervous system (CNS).

4. **Cytokine-regulation model.** Carriage of the MS-associated HLA genotype
entails high-level production of pro-inflammatory Th1-type cytokines.

5. **Aberrant-expression model.** Polymorphisms in promoter regions of classical
HLA genes directly induce the local over-expression of the molecules
encoded by the genes in the context of MS-related inflammation.

6. **LD model.** Non-HLA genes linked to the HLA complex confer susceptibil-
ity to MS, through the actions, or inaction, of their protein products.

Given the great number of HLA associations reported in MS—and the allelic
and locus heterogeneity it implies—it is not unlikely that more than one of these
mechanisms contributes to the pathogenesis of the disease. Indeed, in our own study
cited above (35), the dominant mode of action of DR15, on the one hand, and
the recessive mode of action of the more weakly associated specificity DR17, on the
other, suggest the workings of a complex, two-mechanism model. To explain the mul-
tiple HLA class II associations in rheumatoid arthritis, Zanelli et al. (37) have in fact
proposed such a model, involving both recessive loss of immune protection and
dominant exacerbation of ongoing inflammation. In addition, the association, in

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**Table 2**  **HLA-DR Genotypes in Multiple Sclerosis**

<table>
<thead>
<tr>
<th>Risk genotype</th>
<th>Reference genotype</th>
<th>Barcellos et al. (34)a OR 95% CI</th>
<th>Modin et al. (35)b OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR15/DR15</td>
<td>DRX/DRX</td>
<td>6.7 4.2–10.7</td>
<td>8.3 4.8–14.5</td>
</tr>
<tr>
<td>DR15/DRX</td>
<td>DRX/DRX</td>
<td>2.7 2.1–3.6</td>
<td>3.0 2.4–3.9</td>
</tr>
<tr>
<td>DR15/DR15</td>
<td>DR15/DRX</td>
<td>2.5 1.7–3.7</td>
<td>2.7 1.6–4.8</td>
</tr>
<tr>
<td>DR17/DR17</td>
<td>DRX/DRX</td>
<td>6.1 2.5–15.2</td>
<td></td>
</tr>
<tr>
<td>DR17/DRX</td>
<td>DRX/DRX</td>
<td>0.93 0.68–1.3</td>
<td></td>
</tr>
<tr>
<td>DR17/DR17</td>
<td>DR17/DRX</td>
<td>6.6 2.6–16.8</td>
<td></td>
</tr>
<tr>
<td>DR15/DR17</td>
<td>DRX/DRX</td>
<td>3.9 2.5–6.1</td>
<td></td>
</tr>
<tr>
<td>DR15/DR17</td>
<td>DR15/DRX</td>
<td>1.3 0.81–2.0</td>
<td></td>
</tr>
<tr>
<td>DR15/DR17</td>
<td>DR17/DRX</td>
<td>4.2 2.6–6.9</td>
<td></td>
</tr>
</tbody>
</table>

aIn 187 multicase and 362 single-case families (containing 808 affected and 1574 unaffected subjects); DRX = not DR15.
bIn 937 sporadic cases and 739 controls; DRX = not DR15 or DR17.

**Abbreviations:** OR, odds ratio; CI, confidence interval.
Japan, of the DPB1*0501 allele with the opticospinal form of MS (38) implicates HLA in the determination of the anatomical distribution of demyelinating lesions. Whatever the model, it has been estimated that the HLA region accounts for no more than 15% to 50% of the total genetic risk in MS (39).

**Association**

Association studies are hampered, from the outset, by the difficulty in selecting appropriate candidates from a genome comprised of over 30,000 genes. In general, the genes that have been studied by association analysis in MS have been either functional candidates, chosen on account of the presumed role of the molecules they encode in, e.g., the sequence of immune-cell interactions that culminates in inflammatory demyelination; or positional ones, chosen on account of their location in regions positive for linkage in genome-wide screens. Among the dozens of non-HLA candidates, both functional and positional, studied thus far in case–control datasets in MS (40), none has been consistently shown to be associated with the risk of developing MS. Typically, an initial report of association, published in a high-impact journal, is followed by a less publicized train of negative reports, as has been the case with studies of the genes encoding myelin basic protein (41–44) and CD45 (45–48), as well as a score of others (49). This phenomenon has also plagued association studies in other genetically complex disorders (50).

In a recent review, Colhoun et al. (51) identified three main reasons for this pervasive inability to replicate genetic associations: the failure to attribute findings to chance, publication bias, and inadequate sample size. The authors propose the following remedies for these problems: a more stringent threshold for the declaration of statistical evidence of association—specifically, a reduction of the probability value indicating significance from the traditional $5 \times 10^{-2}$ to the more Bayesian $5 \times 10^{-5}$; internet-based reporting of the results of negative studies; and, for replication studies, sample sizes large enough to detect or exclude effects somewhat smaller than those reported in previous positive studies. They also remark that the prior probability of association will increase when candidate genes are chosen based on functional and positional information, and when the frequencies of common haplotypes are preferentially investigated.

The last two recommendations have been taken to heart in two recent studies of candidate genes in MS. Barcellos et al. (52) investigated, by family-based association analysis, 47 single-nucleotide polymorphisms (SNPs) located in 34 genes encoding proteins involved in inflammatory pathways in two independent datasets of American MS patients and controls. Association in an initial dataset between an SNP in exon 10 of the NOS2A gene on chromosome 17q11 and the risk of MS was confirmed in a second dataset, and subsequent analysis of common haplotypes containing this SNP further corroborated the association. NOS2A was selected as a plausible functional candidate in this study; it encodes the inducible isoform of nitric-oxide synthase, an enzyme that might contribute to MS pathogenesis through the production of neurotoxic oxygen radicals (53,54). In a second study, with a similar multi-stage design, Zhang et al. (55) genotyped 123 SNPs in 66 genes selected on the basis either of their location in regions linked to MS or other autoimmune diseases, or of the potential functional role of their protein products in MS. They ultimately identified one predispositional and one protective five-SNP haplotype spanning the IL7R gene on chromosome 5p13. IL7R encodes the interleukin 7 receptor; signaling via the receptor induces somatic recombination of
the T cell-receptor and immunoglobulin genes, which in turn promotes the proliferation and survival of T and B lymphocytes (56).

Despite the appeal of the Bayesian approaches—whereby the careful selection of candidates increases the prior probability of a true association—recent advances in molecular biology, including the sequencing of the human genome and the development of high-throughput genotyping assays, have made possible a novel, anti-Bayesian approach to complex-disorder gene mapping: the genome-wide association study.

GAMES

In October 2003, in a special issue of the *Journal of Neuroimmunology*, 17 research groups from 17 countries published the results of a multi-center genotyping initiative called GAMES. Using a methodology first proposed by Barcellos et al. (57), the groups genotyped pools of DNA samples, collected in their respective countries, from MS cases and controls (and, in six studies, from familial “trios” of patients and their parents) for the same 6000 microsatellite markers located throughout the genome. The theoretical rationale for the initiative was threefold: genome-wide association studies (or “LD screens”) are a more powerful tool than linkage screens for locating genes with small or modest effects in complex disorders (58); sporadic MS cases are more numerous than familial cases and thus easier to ascertain and recruit; and the signals generated by LD screens are topographically more precise than those identified in linkage screens, since the chromosomal segments shared by members of the general population are shorter than those shared by members of the same immediate family (59). The goal of the pan-European design was to identify both “ubiquitous” genes, important for MS susceptibility in all populations, and “domestic” genes, important solely in a single population. Below we give a brief outline of the datasets examined and the chromosomal regions identified in each of the GAMES screens.

**U.K. GAMES (cases and controls, familial trios):** In an initial report (60), of the ten microsatellite markers exhibiting the greatest evidence of association, three were located in the HLA region (“providing a positive control for the method”), four in regions identified in the first U.K. linkage screen (two on chromosome 17q, and one each on chromosomes 1p and 19q); and three in novel regions (on chromosomes 1q, 2p, and 4q). In a second “refined” analysis (61), in which patients and parents in a subset of trios were individually genotyped for the 529 most promising markers, only the three HLA-region markers were confirmed as associated with MS.

**Australia GAMES (62) (HLA-DRB1*1501-positive cases and unselected controls):** Evidence of association was uncovered for a total of seven markers—four located in regions identified in earlier linkage screens (on chromosomes 12q15, 16p13, 18p11, and 19p13), and three in novel regions (on chromosomes 11q12, 11q23, and 14q21)—suggesting the possibility of interactions between these loci and the HLA locus. An interaction of this kind, between HLA-DR15 and an allele in the promoter of the gene encoding CTLA-4 on chromosome 2q33, was recently described (63).

**Belgium GAMES (64) (cases and controls):** The 20 most promising markers included three located in the HLA class II region and one in the HLA class I region. In addition, the regions identified by the remaining markers contained a number of attractive candidate genes, including the gene encoding the integrin ligand EDIL3 (on chromosome 5q14) and the gene encoding the B-cell-specific transcription factor POU2AF1 (on chromosome 11q23).
Finland GAMES (65) (cases and controls): A total of 108 markers displayed evidence of association. Five chromosomal regions (1q43, 2p16, 4p15, 4q34, and the HLA region on 6p21) contained two or more markers within a 1-Mb interval. In addition, evidence of association was found for a marker located on chromosome 19p13.3, in proximity to the gene encoding ICAM-1. Earlier studies have reported an association between a nonsynonymous SNP in exon 6 of ICAM1 and the risk of MS in case–control datasets from Poland (66) and Finland and Spain (67), but not in datasets from Holland (68) or Sweden (55).

France GAMES (69) (cases and controls, familial trios): After a two-step validation process, involving re-typing of both pooled and individual samples for the 117 most promising markers, two HLA-region markers and five markers from non-HLA regions (two on chromosome 14q32, and one each on chromosomes 7q34, 12q21, and 21q21) displayed evidence of association.

German GAMES [HLA-DRB1∗1501-positive cases and unselected controls (first screen); cases and controls, familial trios (second screen)]: In the first screen (70), association with seven markers (two located on chromosome 1p36, and one each on chromosomes 2q34, 3p25, 4q28, 5q14, and 10q21) was confirmed by individual typing. In the second screen (71), evidence of association was found for two HLA-region markers and nine markers from non-HLA regions. Five of the non-HLA markers were located in regions identified in earlier linkage screens (on chromosomes 2q24, 6p25, 11q23, 12q13, and 19q13), while the remaining four were located in novel regions (on chromosomes 2q33, 15q24, 17p13, and Xq13). Six genes mapping to the region of the most promising marker (on 11q23) encode molecules involved either in the activity and protection of neurons (SCN2B and UBE4A) or in immune homeostasis (CD3D, CD3E, CD3G, and IL10RA).

Hungary GAMES (72) (cases and controls): Of the 33 markers exhibiting evidence of association, six were located in non-HLA regions identified in earlier linkage screens (two on chromosome Xp, and one each on chromosomes 3p14, 5p15, 7p13, and 7q21), and the rest in novel non-HLA regions.

Iceland GAMES (73) (cases and controls): Of the six 2-Mb regions harboring at least two associated markers, three (3q25, 19q13, and the HLA region on 6p21) contained more than one of the 20 most strongly associated markers.

Ireland GAMES (74) (cases and controls): Of the 22 markers displaying evidence of association, three were located in the HLA region. Association with one of the remaining markers, D11S1998, was confirmed by individual typing. The marker maps to a region on chromosome 11q23—the most promising region in the German GAMES screen—which contains the candidate genes IL10RA and CD3E.

Italy GAMES (75) (cases and controls, familial trios): None of the 14 most promising markers mapped to the HLA region. After refined laboratory and statistical analysis, only one of these markers retained evidence of association. This marker, D2S367, maps to a region on chromosome 2p22 that contains several candidate genes encoding molecules involved in apoptotic pathways, including CARD12. It has been reproducibly demonstrated that allelic variants of a gene encoding another member of the CARD family, CARD15, are associated with susceptibility to another putatively autoimmune disorder, Crohn’s disease (76).

Scandinavia GAMES (77) (cases and controls): In two independent screens of pooled samples from Danish, Norwegian, and Swedish cases and controls, nine markers from eight chromosomal regions (1p33, 3q13, 6q14, 7p22, 9p21, 9q21, Xq22, and the HLA region on 6p21) were associated with MS in both screens. Chromosome 1p33 was positive for linkage in the British and Canadian linkage screens.
Poland GAMES (78) (cases and controls, familial trios): The screen identified five associated markers from five different chromosomal regions (2p16, 3p13, 7p22, 15q26, and the HLA region on 6p21). The region on 7p22 contains a candidate gene encoding the apoptosis-related protein CARD11.

Portugal GAMES (cases and controls): In the first of two separate screens (79), evidence of association was found for ten markers from seven chromosomal regions. Three of these regions (5q13, 7q21, and the HLA region on 6p21) were identified in earlier linkage screens and two in earlier GAMES screens (4q35 in the British screen, and 11p15 in the first German screen). The remaining two regions (10p13 and 11q14–24) were novel. In the second screen (80), 46 markers displayed evidence of association. Three chromosomal regions (6q14, 7q34, and the HLA region on 6p21) contained at least two associated markers within a 1.5-Mb interval.

Sardinia GAMES (81) (cases and controls, familial trios): Five markers (from regions on chromosomes 2q36, 6p25, 6p21, 7p12, and 16p12) displayed evidence of association in both cases and controls and familial trios. The marker on 6p21 (D6S271) is located at more than 10 cM from the HLA region.

Spain GAMES (82) (cases and controls): After repeated typing of the 1269 most promising markers, clusters of associated markers were identified on virtually every chromosome. Of the 25 markers with the lowest probability values, seven mapped to the HLA region, while five (on chromosomes 5p15, 5q14, 12q23, 16p13, and 17q23) were identified in earlier linkage screens.

Turkey GAMES (83) (cases and controls): Evidence of association was demonstrated for 12 markers, one of which was located in a region (on chromosome 5p15) identified in the Turkish linkage screen. This region is also homologous with a murine susceptibility locus in experimental allergic encephalomyelitis, an animal model of MS.

In summary, over 80% of the GAMES screens uncovered associations with one or more markers located in the HLA region. In an editorial in the same special issue of the Journal of Neuroimmunology (84), Barcellos and Thomson conclude that the GAMES results “further underscore the universality” of the HLA association in MS. They also point out that a region on chromosome 19q13 was identified by no fewer than seven of the GAMES groups. This region harbors the APOE gene (see below) and was identified as the most promising non-HLA locus in an early meta-analysis of the first four MS linkage screens (85).

Yeo et al. (61), reporting the results of the refined analysis of the British GAMES screen, offer a critical re-appraisal of the basic design of the GAMES project. The power of the GAMES screens to identify MS susceptibility genes, the authors write, was limited by three important factors:

First, the sample sizes used in GAMES are far too small. The pools in each GAMES screen contained DNA from approximately 200 individuals (MS patients, healthy controls, or unaffected parents). Samples of this size provide only modest power to detect strong genetic signals, such as those emanating from markers in the HLA region, and virtually no power at all to detect any weak signals emanating from non-HLA regions.

Second, pooling methodology further reduces the effective size of the samples. Error in estimating allele-frequency differences between affected and unaffected subjects can be divided into sampling error (random noise in a finite sample) and measurement error (noise related to the precision of the method). Sampling error decreases with increasing sample size, but measurement error does not. As Carlson et al. (86) have recently pointed out, for an allele conferring a 1.5-fold increase in the
disease risk with a frequency of 10%, the expected difference in allele frequency between cases and controls is only 4.3%. As the measurement error introduced by pooling is about ±2%, differences of this size could easily be missed, particularly in a genome-wide LD screen, in which corrections for multiple testing must also be performed.

Third, the number of markers used in the GAMES initiative is far too low. The issue of marker density is of course related to the extent of LD throughout the genome, as markers are chosen on account of their presumed proximity to functional polymorphisms. At the time the project was designed, it was believed that LD in the European population was far greater than we now know it to be, and that the entire genome could be screened for association through the use of 6000 microsatellite markers. It turns out, however, based on current estimates of LD, that each GAMES screen tested no more than about 1% of the genome. According to Yeo et al. this last factor—the overestimation of LD and the resulting miscalculation of the required number of markers—represents the greatest shortcoming of the ambitious GAMES initiative.

It is certainly unfortunate that 99% of the genome was left unexplored by the GAMES project. But it is equally problematic that nearly all of the dozens of non-HLA markers “displaying evidence of association” in the 1% of the genome that was explored—markers that now “require confirmation in further studies,” in the words of one of the GAMES groups (69)—are, in light of the great number of statistical tests performed in each screen, presumably false positives (87).

**EXPRESSIVITY**

Although it has proven difficult to identify non-HLA susceptibility genes in MS, or even to determine the precise location of the well-established HLA association, there is little doubt that the risk of developing MS is at least in part genetically determined. In addition, there is now growing evidence that MS expressivity—the variability of the MS phenotype—is also influenced by heritable factors. Intrafamilial concordance in MS has been reported with regard to disease course (88), rate of progression (89), and ultimate disability (90,91), as well as age (92) and clinical manifestations (93) at disease onset.

Moreover, Weinshenker (94) has argued that MS is merely the arbitrarily demarcated prototype for a motley collection of “idiopathic inflammatory demyelinating diseases of the CNS” of varying severity and chronicity—including, at one end of the spectrum, monophasic, multifocal entities such as Devic’s syndrome and, at the other, bout-less myelopathies of dubious dissemination in space or time. Although these “IIDDs” share many features, including presumed immune-mediated pathogenesis, the propensity to develop one rather than the other seems, in some cases, to depend on ethnic background or immunogenetic phenotype; e.g., Devic’s syndrome is more common in Orientals than in Caucasians (95,96), while acute monosymptomatic optic neuritis is more likely to be a manifestation of “prototypic MS” in carriers of HLA-DR15 than in noncarriers (97,98). The genes encoding the β chain of HLA-DR and other classical HLA proteins do not appear to influence MS prognosis, although the results of the innumerable studies that have investigated this phenomenon during the past three decades have been somewhat discordant [reviewed in (99)].

As Kantarci et al. (100) have pointed out, the hunt for disease-modifying genes in MS should make use of long-term outcome measures—such as the “conversion”
from one IIDD to another, or the radiological or histopathological assessment of ultimate disease burden—which are more likely to be influenced by genetic factors than short-term, clinical measures of “stochastic” variables such as relapse frequency or early disability. Indeed, Fazekas et al. (101) have demonstrated the superiority of magnetic resonance imaging (MRI)-related outcome measures, in the context of genetic studies of MS expressivity, to measures based on clinical disability scales: in their initial dataset of 83 patients, the APOE ε4 allele was significantly associated with greater lesion burden on MRI, whereas a significant effect on disability as assessed by the Expanded Disability Status Scale was not observed until the dataset was expanded to include 374 patients (102). Indeed, in the 25 studies examining the effect of APOE ε4 on MS prognosis published to date (Table 3), 10 of the 18 studies employing clinical measures of disease severity have been negative (102–112,115–118,122,124,125), while four of seven studies incorporating radiological measures have been positive (101,113,114,119,120,122,123).

APOE encodes apolipoprotein E, a molecule synthesized and secreted by glial cells that serves as a ligand mediating the uptake of plasma lipoproteins, which are vital for membrane repair. The ε4 allele is associated, clinically, with susceptibility to, and lower age at onset in, both familial and sporadic Alzheimer’s disease (AD) (126) and adverse outcome following head trauma and stroke (127); pathologically, with less efficient dendritic remodeling in brains from AD patients (128); and, radiologically, with greater T1-weighted lesion load on MRI in patients with MS (101). APOE alleles could conceivably influence clinical outcome in MS through the differential effects of the isoforms they encode on remyelination or axonal degeneration. Chapman et al. (129) have hypothesized that such effects could be the mechanism behind both the progression-hastening impact of APOE ε4 in neurodegenerative disorders diagnosed early in life, such as MS and Creuzfeldt-Jakob disease (130) and the allele’s onset-hastening impact in neurodegenerative disorders diagnosed late in life, such as AD and Wilson’s disease (131).

When all the evidence is weighed (Table 3), polymorphism of APOE appears to explain at least a portion of the radiological and clinical heterogeneity of MS. Still, the most meaningful form of heterogeneity in MS may prove to be that described by Lucchinetti et al. (132), who observed four distinct patterns of MS pathology—with each pattern occurring alone in a given subject—in biopsy or autopsy material from 83 patients. These pathological patterns may represent “proximal phenotypes”—upstream biological determinants of an indeterminate clinical phenotype—of the type whose identification and analysis (133) have been advocated by Terwilliger and Göring (133) for the successful genetic dissection of etiologically heterogeneous complex diseases. As Kantarci et al. (100) remark, however, only after laboratory or neuroimaging correlates of the patterns are defined will it become possible to routinely analyze the contribution of genetic factors to the pathological heterogeneity of MS. In the meantime, most MS expressivity studies to date (100) have had to content themselves with more distal clinical and paraclinical surrogates.

PROSPECTS

The investigation of proximal phenotypes [also called “endophenotypes” or “intermediate phenotypes” (134)], which are presumed to be genetically less heterogeneous than conventionally defined downstream “disorders,” may be one way of overcoming the difficulties encountered to date in attempts to map susceptibility genes in
### Table 3  
Studies Assessing Impact of *APOE* on Disease Severity in Multiple Sclerosis

<table>
<thead>
<tr>
<th>References</th>
<th>Country</th>
<th>Polymorphism(s)</th>
<th>Dataset</th>
<th>Severity assessment</th>
<th>Authors’ conclusions</th>
<th>Adverse effect of ε4?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evangelou et al. (103)</td>
<td>U.K.</td>
<td>ε2–4</td>
<td>95 MS</td>
<td>Dichotomization based on EDSS and duration</td>
<td>ε4 associated with more aggressive course</td>
<td>Yes</td>
</tr>
<tr>
<td>Ferri et al. (104)</td>
<td>Italy</td>
<td>promoter-491, ε2–4</td>
<td>161 RRMS</td>
<td>Dichotomization based on EDSS and duration</td>
<td>Polymorphisms not associated with clinical burden over time</td>
<td>No</td>
</tr>
<tr>
<td>Oliveri et al. (105)</td>
<td>Italy</td>
<td>promoter-491, ε2–4</td>
<td>89 BOMS</td>
<td>Dichotomization based on results of neuropsychological testing</td>
<td>Promoter AA genotype associated with cognitive impairment</td>
<td>No</td>
</tr>
<tr>
<td>Hamilton et al. (106)</td>
<td>U.K.</td>
<td>ε2–4</td>
<td>265 MS</td>
<td>Progression index (EDSS/duration in years)</td>
<td>No association between gene variants and progression index</td>
<td>No</td>
</tr>
<tr>
<td>Weatherby et al. (107)</td>
<td>U.K.</td>
<td>ε2–4</td>
<td>205 BOMS</td>
<td>Dichotomization based on EDSS after 10 years’ duration</td>
<td>No relationship between genotype frequencies and disease severity</td>
<td>No</td>
</tr>
<tr>
<td>Weatherby et al. (108)</td>
<td>U.K.</td>
<td>ε2–4</td>
<td>50 PPMS</td>
<td>Dichotomization based on raw EDSS or progression index</td>
<td>Outcome not significantly influenced by ε4 possession</td>
<td>No</td>
</tr>
<tr>
<td>Fazekas et al. (101)</td>
<td>Austria</td>
<td>ε2–4</td>
<td>83 MS</td>
<td>T1- and T2-weighted lesion loads on MRI</td>
<td>Morphological support for a negative effect of ε4 on MS</td>
<td>Yes</td>
</tr>
<tr>
<td>Høgh et al. (109)</td>
<td>Denmark</td>
<td>ε2–4</td>
<td>240 MS</td>
<td>Log-transformed progression index</td>
<td>Progression rate faster in ε4 homoygotes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ballerini et al. (110)</td>
<td>Italy</td>
<td>ε2–4</td>
<td>66 BOMS</td>
<td>Time to secondary-progressive phase</td>
<td>ε2 exerts protective role against onset of progressive disease form</td>
<td>No</td>
</tr>
<tr>
<td>Chapman et al. (111)</td>
<td>Israel</td>
<td>ε2–4</td>
<td>205 MS</td>
<td>Time to EDSS 4.0 and 6.0</td>
<td>Compelling evidence for ε4 effect on MS disability</td>
<td>Yes</td>
</tr>
<tr>
<td>Fazekas et al. (102)</td>
<td>Austria</td>
<td>ε2–4</td>
<td>374 MS</td>
<td>Progression index; relapse rate</td>
<td>Strong support for modulation of MS severity by ε4</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*(Continued)*
Table 3  Studies Assessing Impact of *APOE* on Disease Severity in Multiple Sclerosis (*Continued*)

<table>
<thead>
<tr>
<th>References</th>
<th>Country</th>
<th>Polymorphism(s)</th>
<th>Dataset</th>
<th>Severity assessment</th>
<th>Authors’ conclusions</th>
<th>Adverse effect of ε4?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schmidt et al. (112)</td>
<td>U.S.A.</td>
<td>ε2–4</td>
<td>614 MS</td>
<td>Dichotomization based on EDSS and duration; time to EDSS 7.0</td>
<td>ε4 associated with “severe MS” and ε2 with “mild MS”</td>
<td>Yes</td>
</tr>
<tr>
<td>Masterman et al. (125)</td>
<td>Sweden</td>
<td>ε2–4</td>
<td>264 MS</td>
<td>Comparison of opposite septiles of disability-stratified cohort</td>
<td>Odd ratios conferred by ε4 carriage rise in increasingly antipodal quantiles</td>
<td>Yes</td>
</tr>
<tr>
<td>Schreiber et al. (113)</td>
<td>Denmark</td>
<td>ε2–4</td>
<td>70 MS</td>
<td>Progression index; T2-weighted MRI lesion load/duration</td>
<td>No confirmation of association between ε4 carriage and disease progression</td>
<td>No</td>
</tr>
<tr>
<td>Enzinger et al. (114)</td>
<td>Austria</td>
<td>ε2–4</td>
<td>72 RRMS</td>
<td>EDSS; relapse rate; N-acetylaspartate levels on 1H-MRS</td>
<td>In vivo evidence of more extensive axonal damage in ε4 carriers</td>
<td>Yes</td>
</tr>
<tr>
<td>Guerrero et al. (115)</td>
<td>Spain</td>
<td>ε2–4</td>
<td>42 MS</td>
<td>Progression index; relapse rate</td>
<td>No confirmation that ε4 is predictor of disability progression</td>
<td>No</td>
</tr>
<tr>
<td>Niino et al. (116)</td>
<td>Japan</td>
<td>ε2–4</td>
<td>135 BOMS</td>
<td>Progression index; dichotomization based on EDSS after 10 years’ duration</td>
<td>No association between genotypes and disease severity</td>
<td>No</td>
</tr>
<tr>
<td>Savettieri et al. (117)</td>
<td>Italy</td>
<td>promoter-491, ε2–4</td>
<td>428 MS</td>
<td>Progression index</td>
<td>No confirmation of association between ε4 and disease severity</td>
<td>No</td>
</tr>
<tr>
<td>Study</td>
<td>Location</td>
<td>Sample Size</td>
<td>Measures</td>
<td>Results (Alleles)</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>---------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Kantarci et al. (118)</td>
<td>U.S.A.</td>
<td>3 promoter</td>
<td>Progression index; time to EDSS 6.0, time to secondary-progressive phase</td>
<td>ε2 associated with less severe disease in women</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SNPs, ε2–4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>De Stefano et al. (119)</td>
<td>Italy</td>
<td>ε2–4</td>
<td>Normalized brain volume and T2-weighted lesion load on MRI</td>
<td>More pronounced brain damage in ε4 carriers (even in earliest disease stages)</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Enzinger et al. (120)</td>
<td>Austria</td>
<td>ε2–4</td>
<td>Changes in brain volume and in T1- and T2-weighted lesion loads on MRI</td>
<td>More pronounced tissue destruction in ε4 carriers</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Santos et al. (121)</td>
<td>Portugal</td>
<td>ε2–4</td>
<td>Raw EDSS; progression index</td>
<td>Role of ε4 in MS progression may be limited to initial disease stages</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Zakrzewska-Pniewska et al.</td>
<td>Poland</td>
<td>ε2–4</td>
<td>EDSS; extent of demyelination and brain atrophy on MRI</td>
<td>No effect of alleles on clinical or MRI severity parameters</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Zwemmer et al. (123)</td>
<td>Netherlands</td>
<td>ε2–4</td>
<td>Progression index; time to EDSS 6.0; lesion loads and brain volume on MRI</td>
<td>No relation between alleles and clinical or MRI measures of disease severity</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Pinholt et al. (124)</td>
<td>Denmark</td>
<td>ε2–4</td>
<td>Progression index</td>
<td>Faster disease progression in ε4 carriers</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

Supplemental Table 2

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Sample Size</th>
<th>Measures</th>
<th>Results (Alleles)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCases of “Asian-type” MS were excluded.</td>
<td></td>
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</tbody>
</table>

**Abbreviations**: MS, multiple sclerosis; EDSS, Expanded Disability Status Scale; RR, relapsing-remitting; BO, bout-onset (RR or secondary progressive); MRI, magnetic resonance imaging; PP, primary progressive; 1H-MRS, proton magnetic resonance spectroscopy; SNPs, single-nucleotide polymorphisms. 

**Source**: From Ref. 125.
complex diseases. As Carlson et al. (86) have explained, concentrating on proximal phenotypes improves the “signal-to-noise ratio” of any genetic factor contributing to the overall phenotype (provided the contribution is mediated by the proximal phenotype in question).

In this spirit, Kikuchi et al. (135) have recently described two separate “subpopulations” of patients with “Western-type” MS in Japan: in the first, in which women outnumber men by a factor of five to one, MS is associated with the HLA-DR15 and the presence of oligoclonal bands (OCBs) in the cerebrospinal fluid (CSF); in the second, in which women are still more common, but only by a factor of two to one, MS is associated with HLA-DR4 and the absence of OCB. We have now confirmed, in a Swedish dataset, the associations of DR15 and DR4 with, respectively, OCB-positive and OCB-negative MS (136). Moreover, we have also demonstrated that HLA-DR15 is associated with an earlier age at onset in MS (137,138), a finding corroborated in two subsequent studies (139,140). In the latter two studies, the association between DR15 and MS susceptibility was stronger in females than in males. We have also observed that over 80% of our OCB-positive MS patients with an age at onset under 21 years are carriers of DR15 (99). Thus, another aspect of the MS phenotype, age at onset, could perhaps be incorporated into the scheme proposed by Kikuchi et al. (141): early-onset MS cases are typically OCB-positive, DR15-positive females, while late-onset cases are often OCB-negative, DR4-positive males.

This strategy of stratification—based on clinically, paraclinically, demographically, and immunogenetically defined intermediate phenotypes—may in the future facilitate the identification of non-HLA genetic risk factors (or even gender-specific non-genetic risk factors) in MS. Indeed, in recent studies from Japan (141) and Finland (142), after stratification for gender and HLA class II phenotype, genotypes at an intronic SNP in the gene encoding estrogen receptor 1 were shown to confer, respectively, 16- and 19-fold risks for the development of MS in HLA-DR15-positive females. If this association turns out to be reproducible, it would strengthen the suspicion of a hormonal basis for the gender bias in MS; suggest the importance of immunoendocrine crosstalk in MS; imply the existence of separate genetic risk factors in men and women and in carriers and noncarriers of DR15; and, perhaps most importantly, designate a potential target for pharmacological therapy (or prophylaxis).

Another strategy used in genetic studies to decrease the heterogeneity of the MS phenotype is to investigate geographically isolated populations with a high prevalence of MS, or multigenerational families in which MS appears to be inherited in a Mendelian fashion. The rationale behind this strategy assumes that, within each isolated population or within each family displaying Mendelian inheritance, the same combination of genetic and environmental factors—which represents a subset of all the risk factors in the total MS population—is contributing to disease risk.

In 1994, Binzer et al. (143) reported that, in Överkalix, Sweden, 12 of the village’s 4744 inhabitants suffered from MS (corresponding to a prevalence of 253 cases per 100,000 persons); through church archives, it could be shown, in the majority of cases, that the MS patients descended from the same 18th-century ancestral couple. In a subsequent genetic study of this population (144)—a genome-wide screen, followed by analysis of the transmission of alleles within familial trios [by the transmission-disequilibrium test (TDT)]—we found that 12 of 15 affected subjects carried some portion of a conserved haplotype on chromosome 17p11. We later performed genome-wide TDT analysis on MS patients and healthy family members from another geographically isolated population in Värmland County, Sweden (145),
and identified five regions that appeared to be associated with MS (on chromosomes 2q23–31, 6p24–21, 6q25–27, 14q24–32, 16p13–12, and 17q12–24).

In both AD and Parkinson’s disease—which, like MS, are common neurological disorders believed to be caused in the majority of cases by the interaction of several genes with unknown environmental factors—the identification of rare disease forms inherited in a classic Mendelian fashion has helped investigators elucidate generalizable pathogenetic mechanisms. We have recently characterized a consanguineous family of Middle Eastern origin exhibiting multiple cases of MS and performed a genome-wide screen on nine family members now residing in Sweden (146). Based on the presence of consanguinity, our a priori hypothesis was that the disease was being transmitted in an autosomal recessive manner in the pedigree; however, we found no chromosomal region for which all affected family members were homozygous by descent. Yet, there are indications that MS may not be a straightforwardly autosomal recessive trait in our pedigree; e.g., an LOD score of 1.7 was found on the X chromosome, suggestive of an X-linked trait partially penetrant in females.

At the same time, consanguinity is known to increase the likelihood that non-Mendelian, oligogenic traits will occur multiple times within the same family; in a recent genome-wide screen of 16 members of a large inbred Amish kinship, 7 of whom had MS, Vitale et al. (147) reported a maximum LOD score of 2.7, conditional on the presence of HLA-DR15, for a locus on chromosome 12p12, suggesting a two-locus inheritance model in the pedigree. Meanwhile, in another recent genome-wide screen of a seemingly Mendelian multigenerational MS kinship, Dyment et al. (148) found, by parametric analysis, no evidence for linkage to the HLA-DRB1 locus, but, by TDT, significant association with the DRB1*15 allele. The authors conclude, in cliffhanger fashion, that DRB1 “is . . . not the hypothetical single gene acting to determine MS within this family,” but rather a “modifier” of either penetrance or some unnamed phenotypic trait.

It is important to note that it is uncertain to what extent loci identified in population isolates or Mendelian families will be of relevance to sporadic MS cases from the general population. Indeed, according to Terwilliger et al. (149), this is the central paradox of complex-disorder gene mapping: the simpler one makes the localization of susceptibility genes, the more difficult it becomes to estimate the contribution of any localized genes to the total risk of the disease in question. Indeed, there is growing consensus that the future of gene mapping in complex disorders lies, not in the investigation of upstream phenotypes or exceptional pedigrees, but rather in SNP-based genome-wide association studies of large datasets of unexceptional cases and controls.

It has been estimated that there are 15 million SNPs in the human genome (150). There is agreement among researchers that only a subset of these SNPs needs to be genotyped in a genome-wide LD screen, but there is disagreement regarding the best way to select this subset (151). Proponents of “map-based” screening favor a subset of anonymous SNPs, each in LD with a highly conserved ancestral haplotype (152), while proponents of “sequence-based” screening favor SNPs with functional consequences, such as those that encode amino-acid substitutions or disrupt splice sites (153). The relative merits of the two approaches have been outlined in a recent review (150).

At the same time, Merikangas and Risch (154) have argued that, from a public-health standpoint, not all complex disorders are worthy of the “expensive and laborious tools of molecular genetics.” They propose that, with regard to the mapping of germline variants that increase disease risk, complex disorders that are “highly
amenable to environmental modification”—such as nicotine dependence and AIDS—should be given much lower priority than disorders for which the hypothesized environmental risk factors remain unknown, such as schizophrenia and MS. However, given the recent breakthroughs in MS epidemiology—in particular, findings regarding the influence of smoking (155,156), sunlight exposure (157,158), vitamin D intake (159), and the immune response to common herpes viruses (160,161) on disease susceptibility—it is uncertain how long MS will remain in the latter, prioritized category.

Keeping abreast of all the genetic studies performed in MS—many of them underpowered or otherwise flawed in their design, and nearly all of them inconclusive—has been rightly described as “a Sisyphean task” (51). Still, we agree with Sawcer and Compston (162), the principal instigators of the GAMES initiative, that the current state of MS genetics is a “cup half full,” rather than a “cup half empty.” To paraphrase the Belorussian Talmudist Saul Lieberman, although futility is futility, the history of futility is scholarship.

REFERENCES


Evidence for an Infectious Etiology of Multiple Sclerosis

Stuart D. Cook
Department of Neurology/Neurosciences, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey, U.S.A.

INTRODUCTION

Multiple sclerosis (MS) is an acquired inflammatory disease of the central nervous system (CNS) of uncertain etiology. On the basis of the available evidence, it seems probable that MS is a complex disease in which exposure to one or more environmental agents predisposes genetically susceptible individuals to develop immunologically mediated CNS demyelination and axonal injury. In terms of the environment, the degree or type of exposure to solar ultraviolet radiation, smoked meat, vitamin D, vaccines, organic solvents, cigarette smoking, cold damp weather, workplace environment, and stress have all been suggested as predisposing to MS; however, it is likely on epidemiologic considerations that any such contribution is probably secondary rather than primary (see Chapter 1). There is also considerable indirect support for the role of infection in initiating and perhaps perpetuating the inflammatory pathology that results in neurologic symptoms and disability (1,2). Over the past decade, several novel exogenous agents have been identified in MS brain or cerebrospinal fluid (CSF) (3–5), raising the possibility that antiviral or antibacterial drugs could alter disease prognosis. While the specificity of these findings is in doubt, interest in an infectious cause of MS remains strong.

This chapter comprises a critical review of evidence for and against an infectious etiology of MS.

HISTORICAL PERSPECTIVE

The concept that MS may be caused or aggravated by an infectious agent is not new. Although both Charcot and Leyden suggested a relationship between an antecedent illness and the onset of MS, Pierre Marie more formally raised this possibility shortly after the clinical and pathological characteristics of MS were initially defined (6,7). In a paper published in 1884, Marie states “I was struck by the coincidental occurrence of MS with infectious illnesses and by the close relationship that, from a theoretical point of view unites these two afflictions; thus, I made an effort to renew
the idea that MS often starts as an infectious process . . . ” Because the initial phase of MS is often subclinical and the precise onset is difficult to determine, Marie was probably referring to the well recognized occurrence of MS exacerbations associated with acute bacterial or viral infections.

Over the past century, numerous, often highly publicized claims of isolation or identification of viruses, bacteria, and spirochetes from or in MS tissue have been made (8–10). Unfortunately, independent attempts at verifying these reports or determining their specificity have been generally unsuccessful, leading to a pervasive skepticism over subsequent claims of linkage between MS and infectious agents. Nevertheless, suspicion remains high that an infectious agent is responsible for initiating the as yet imprecisely defined sequence of immunological and inflammatory events that lead to CNS demyelination in this enigmatic disease (Table 1).

In recent years, with the advent of sophisticated molecular technology and the general failure of traditional isolation and culture techniques to identify conventional organisms, attention has been directed more toward unusual pathogens that lead to chronic latent infection or agents which trigger MS but may not persist in the host. Viruses can cause demyelinating disease in animals and humans, remain latent in neural tissue for extended periods, and cause chronic persistent infections of the CNS-characteristics attractive for a putative MS pathogen. However, the recent demonstration that bacterial or bacteria-like organisms are responsible for cat scratch disease (Rochalimaea species), peptic ulcer (Helicobacter pylori), chronic Lyme arthritis (Borrelia burgdorferi), Whipple’s disease (Tropheryma whippelii), and other diseases previously considered “idiopathic,” should leave the reader with an open mind as to the possible microbial spectrum of MS precipitants. In Whipple’s disease, the bacterium, T. whippelii, was first cultured almost 100 years after the disease was initially described. While several reasons could be put forth to explain the delay in identifying this elusive organism, it is relevant that the agent has to be grown intracellularly in the absence of antibiotics and takes an extremely long time to grow (11). In this regard, Blaser (12) has pointed out that many infectious diseases have not been linked to their causative agent in a timely fashion, even in the modern era, because of technical barriers, including fastidious culture requirements, low

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Negative, Unconfirmed, or Controversial Studies for an Infectious Agent or Genome in Multiple Sclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>MS-associated agent</td>
</tr>
<tr>
<td><em>Borrelia burgdorferi</em></td>
<td>Measles virus</td>
</tr>
<tr>
<td>CDV</td>
<td>Mumps virus</td>
</tr>
<tr>
<td><em>Chlamydia pneumonias</em></td>
<td>Papovavirus</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>Parainfluenza virus</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>Rabies virus</td>
</tr>
<tr>
<td>EBV</td>
<td>Retrovirus</td>
</tr>
<tr>
<td>HHV-6</td>
<td>Rubella</td>
</tr>
<tr>
<td>HTLV-1 and/or 2</td>
<td>Scrapie agent</td>
</tr>
<tr>
<td>Herpes simplex 1 virus and/or 2</td>
<td>Simian virus</td>
</tr>
<tr>
<td>HIV</td>
<td>SMON-like virus</td>
</tr>
</tbody>
</table>

*Abbreviations:* CDV, canine distemper virus; EBV, Epstein–Barr virus; HIV, human immunodeficiency virus; HHV, human herpes virus; HTLV, human T-cell leukemia/lymphoma virus; SMON, subacute myelo-optic neuropathy.

*Source:* Modified from Ref. 1.
tissue concentrations of the pathogen, or conceptual barriers, including the failure to recognize that the disease could be due to an uncommon complication of a common microbe or because of a long latent period between infection and disease expression. Clearly, the same issues need to be considered in MS.

**EVIDENCE FOR AN INFECTIOUS ETIOLOGY**

It seems safe to say that no proof yet exists for a specific exogenous cause of MS; however, a growing body of evidence suggests that one or more pathogens trigger MS. This evidence is based on the studies of MS epidemiology, concordance rates in twins, CNS pathology, and laboratory findings, as well as the existence of viral models of demyelinating disease.

**Epidemiology**

One of the major clues to MS etiology comes from analysis of the remarkable worldwide pattern of MS. This shows a crude but inconsistent north–south gradient in North America and Europe; a lower prevalence in most of Asia, Africa, and South America (although many of these studies are less than definitive because of uncertainty about the completeness of case ascertainment); and a reverse south–north gradient in Australia and New Zealand (Chapters 1 and 2). This nonrandom pattern is different from that seen with many other acute or chronic “autoimmune” diseases of the central and peripheral nervous systems (PNS), such as acute disseminated encephalomyelitis (ADEM), the Guillain-Barré syndrome (GBS), and chronic inflammatory demyelinating polyneuropathy (CIDP); however, a similar worldwide pattern can be seen for type 1 diabetes in Europe and other allergic or “autoimmune” disorders such as Crohn’s disease are not randomly distributed (15).

When unusual worldwide patterns of the disease are seen, interest heightens the potential for identifying causative mechanisms. Some diseases with characteristic geographical features are genetic in origin. This would include Tay-Sachs disease, affecting primarily Ashkenazi Jews; thalassemia, occurring in populations originating in southern Italy, other Mediterranean countries, Africa, and Asia; and sickle cell disease in individuals with African ancestry. Other diseases caused by specific infectious agents may have a unique distribution because of environmental factors, including cultural characteristics of the population and degree of exposure to the vectors involved in transmission. Rabies and some parasitic diseases can be considered in this category. Yet other diseases—such as tuberculosis, paralytic poliomyelitis, and rheumatic heart disease—may have a restricted global pattern owing to a combination of environmental factors, including poor hygiene or crowded conditions, and genetic predisposition. Which of these possibilities best fits MS is debatable, but on the basis of available evidence, the latter two seem more likely than the former.

There is also a consistent but not invariable effect of migration in altering MS risk in migrants or their offspring, depending on age at migration and whether the movement is from high- to low-risk regions or the converse (Chapters 1 and 2). The effect of migration on disease risk has not been associated clearly with other chronic (immune-mediated) diseases with some exceptions, i.e., type 1 diabetes and asthma (15). For example, the development of both MS and type 1 diabetes in the offspring of immigrants from the Indian subcontinent who migrate to the United Kingdom is the same as the endogenous British population (15,16).
Further evidence for an infectious agent comes from controversial reports of changes in MS incidence, up or down, or even frank clustering in some locales, suggesting that MS is not always a stable endemic disease as would be predicted for a purely genetic disorder. In addition, MS patients often have measles, EBV, or other childhood infections at a later age than controls (17–23). Whether this indicates that late exposure to multiple, a few, or a single pathogen is a critical factor in triggering MS remains to be determined. Others have not confirmed or criticized these studies as the retrospective determination of date of childhood infections may be inaccurate due to the long time which has elapsed or because of recall bias (24).

Twin Studies

Although genetic factors also seem important in determining MS susceptibility (see Chapter 2), the Canadian MS twin study showed a concordance rate of only 31% in monozygotic pairs when compared with less than 5% in dizygotes, even with magnetic resonance imaging (MRI) brain scans to detect subclinical disease, and long-term follow-up evaluations (25). This means that in over two-thirds of identical twins, both twins do not develop MS, even when one of the pair does. It seems safe to conclude from this evidence that in most instances genetic factors alone are insufficient to cause MS. It is also of interest that the concordance rate for type 1 diabetes, which shares a similar geographic distribution to MS, is 33% in monozygotic twins. A remarkably similar concordance rate in monozygotic as compared to dizygotic twins is also seen in paralytic poliomyelitis (26). Although genetic factors may have determined who develops paralysis following a polio virus infection, the key to preventing the disease was identifying the viruses responsible and developing an effective vaccine. The same may yet prove to be true for MS.

Identical twins share not only genetic sameness but many common environmental exposures including diet, exposure to sunlight, vaccination schedules, and communicable diseases during the first 15 to 18 years of life. Assuming that an infectious agent is important in causing MS, one can speculate that either the agent is not readily spread from twin to twin (i.e., low infectivity, sexual spread, animal vector) or that host factors other than exposure are important (i.e., dose of infectious agent, route of infection, status of the individual’s immune system). The relatively low concordance rate in identical twins, narrow age range for onset of MS, restricted geography, and migration effects appear to the author to be more suggestive of one or a few agents causing MS rather than a large number, as many experts believe.

CNS and CSF Studies

CNS inflammatory lesions, abnormal profiles of chemokines and cytokines, and other effector molecules in brain, blood, and CSF; alterations in T- and B-cell subset concentrations (Chapters 4 and 8) as well as CSF changes in immunoglobulin G (IgG) and free light chains levels, including the presence of electrophoretically restricted oligoclonal bands (OCBs) in MS (Chapters 4 and 8) are all compatible with the effect of either an infectious agent or an autoimmune process. Similar pathological changes can be seen with viral and nonviral encephalitides as well as in the animal model experimental allergic encephalomyelitis (EAE). Likewise, the CSF IgG abnormalities seen in MS are mirrored in many infectious disorders including subacute sclerosing panencephalitis (SSPE), neurosyphilis, Lyme disease, and viral encephalitis as well as in autoimmune disorders such as EAE (24–30). Whereas
in most viral infections OCBs react with or can be adsorbed by disease-specific viral antigens (28,31,32), attempts at removing MS oligoclonal bands after exposure to candidate agents have generally been unsuccessful (25,28) or, if positive (33), remain unconfirmed. It is currently unclear whether the OCBs in MS CSF react to as yet undefined specific infectious agents or to host antigens.

**Infectious Agents Causing Demyelination**

Clues to the etiology of MS might come from viruses and other infectious agents capable of causing spontaneous demyelination in humans or animals (Table 2). Several DNA and RNA viruses can produce inflammatory myelin loss in the CNS or PNS. In humans, infection with measles, EBV, varicella, and other pathogens can result in ADEM or postinfectious encephalomyelitis (1), whereas infections with *Campylobacter jejuni*, EBV, *Mycoplasma pneumoniae*, and cytomegalovirus (CMV) are often associated with GBS (34). However, acute infection with these agents does not usually produce recurrent or chronic demyelination, suggesting that a persistent infection, host factors, or as yet unidentified agents are responsible for causing MS and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). Persistent infection with other viruses—including papovavirus (progressive multifocal leukoencephalopathy), human T-cell leukaemia/lymphoma virus type 1 (HTLV-1; tropical spastic paraparesis), and human immunodeficiency virus (HIV)—result in chronic demyelination, although there are distinct pathological differences between these chronic encephalitides and MS. In contrast, canine distemper virus (CDV) infection in dogs, Theiler’s murine encephalomyelitis virus, and coronavirus infections in mice, and other animal viruses can cause demyelination in their hosts, similar to MS, with an acute, exacerbating, or progressive course.

**Serological Studies**

Serological studies of MS serum and CSF show increased antibody titers to EBV, measles, and CDV as well as to other infectious agents when compared with controls (Table 3). Using a variety of techniques, serum antibodies from MS sera are elevated to multiple EBV, measles, and CDV peptides. The viral antibody titer increases are usually modest (up to a fivefold increase for EBV vs. a twofold increase for measles). Increases in viral antibody titers have been reported to numerous other agents, including vaccinia, herpes zoster, rubella, mumps, herpes simplex, adenovirus, para-influenza 2, and influenza viruses. Although any agent inducing a consistent increase

<table>
<thead>
<tr>
<th>Human</th>
<th>Animal</th>
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<tbody>
<tr>
<td>ADEM</td>
<td>CDV</td>
</tr>
<tr>
<td>Progressive multifocal leukoencephalopathy</td>
<td>Murine coronaviruses</td>
</tr>
<tr>
<td>HIV encephalopathy</td>
<td>Theiler’s virus</td>
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<tr>
<td>HTLV-1</td>
<td>Visna virus</td>
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<tr>
<td>Other</td>
<td>Other</td>
</tr>
</tbody>
</table>

*Abbreviations:* ADEM, acute disseminated encephalomyelitis; CDV, canine distemper virus; HIV, human immunodeficiency virus; HTLV, human T-cell leukaemia/lymphoma virus.  
*Source:* From Ref. 1.
in antibody levels in MS patients could be considered a potential causal agent, other reasons for increased antibodies need to be considered. For example, reactivation of a latent virus secondary either to the MS inflammatory process or to the use of immunosuppressive drugs could in some instances explain these serological findings. Alternatively, elevated antibody levels to multiple infectious agents in the same patient could be attributable to nonspecific generalized B-cell hyperactivity. Such a phenomenon could also explain the presence of OCBs in MS CSF.

In summary, the occurrence of spontaneous human and animal models of virus induced demyelination as well as evidence from epidemiological, serological, and pathological studies provides strong support for the existence of an infectious trigger but not as yet for a persistent CNS infection.

### POSSIBLE MECHANISMS OF VIRUS-INDUCED DEMYELINATION

If one assumes that MS is triggered by an infectious agent, at least two mechanisms by which the infectious agent could cause tissue injury can be considered (1,14,35).

#### Persistent Infection

The direct infection hypothesis implies that the virus persists in the brain or perhaps in other organs, periodically seeding the brain. There are many examples of persistent

### Table 3: Higher Serum or Cerebrospinal Fluid Antibody Titer to Pathogens in Multiple Sclerosis Patients Than Controls

<table>
<thead>
<tr>
<th>Serum Pathogen</th>
<th>CSF Pathogen</th>
</tr>
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<tbody>
<tr>
<td>Adenovirus</td>
<td>Measles virus</td>
</tr>
<tr>
<td>CDV</td>
<td>Mumps virus</td>
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<tr>
<td>EBV</td>
<td>Parainfluenza virus</td>
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<tr>
<td>Herpes simplex</td>
<td>Rubella virus</td>
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<tr>
<td>HHV-6</td>
<td>Vaccinia virus</td>
</tr>
<tr>
<td>Influenza</td>
<td>Varicella zoster virus</td>
</tr>
<tr>
<td>Chlamydia pneumonia</td>
<td>Measles virus</td>
</tr>
<tr>
<td>CMV</td>
<td>Mycoplasma pneumonia</td>
</tr>
<tr>
<td>EBV</td>
<td>Parainfluenza 1, 2, and 3</td>
</tr>
<tr>
<td>HHV-6</td>
<td>Respiratory syncytial virus</td>
</tr>
<tr>
<td>Herpes simplex</td>
<td>Rubella virus</td>
</tr>
<tr>
<td>Human coronavirus</td>
<td>Vaccinia virus</td>
</tr>
<tr>
<td>Influenza A and B</td>
<td>Varicella zoster virus</td>
</tr>
</tbody>
</table>

**Abbreviations**: CDV, canine distemper virus; EBV, Epstein-Barr virus; CMV, cytomegalovirus; HHV, human herpes virus.

**Source**: From Ref. 1.
infections or viral latency in the nervous system. In the former category are measles virus (SSPE), HIV, HTLV-1, papovavirus, and rubella virus encephalopathies. Herpes simplex, herpes zoster, EBV, certain retroviruses, and human herpes virus 6 (HHV-6) are examples of common viruses that persist in neural or other tissue, usually in a latent form. In these models, chronic low grade infection, periodic reactivation of latent virus, or seeding of the brain through a hematogenous route could cause direct injury to glial cells or neurons. Alternatively, the agent could initiate an autoimmune response secondary to release or alteration of previously sequestered self-antigens with epitope spread or through molecular mimicry (36,37). In addition, the infectious agent could prime macrophages and lymphocytes, so that subsequently non-encephalitogenic activated T- or B-cells could enter the CNS and release cytokines or antibodies causing demyelination by a bystander effect (37). Lastly, the agent could act as a super-antigen, directly stimulating encephalitogenic T-cell clones (38). Through any of these mechanisms, demyelination and axonal injury could arise.

If the agent does persist in the host, it should ultimately be identifiable, particularly early in the course of the disease using appropriate techniques, including the exquisitely sensitive polymerase chain reaction (PCR) or newer molecular techniques for identifying exogenous genes or proteins. Further, if an infectious agent persists in the patient, it might be possible to show a serological association between pathogen and disease. Antibody to the agent might be extremely elevated when compared with controls, even though the controls had been infected transiently by the same virus. For example, SSPE is a persistent measles virus infection of the brain, leading to very high serum and CSF measles antibody titers to most but not all measles virus proteins. However, even with persistent infection, viral antibody titers are not always elevated. For example, in progressive canine distemper encephalitis, viral antibody titers are often lower in animals with fulminant disease, perhaps related to virus-induced lymphopenia and immunosuppression (1,14).

Although discouraging, the failure to reproducibly culture an organism from or identify viral genome or antigens in MS tissues cannot be considered as proof that an infectious agent is not present (1,9,14). Nevertheless, the negative results to date indicate the need to consider alternative mechanisms for CNS lesion genesis in MS.

**Transient Infection**

The second mechanism that can be considered for infection-induced lesion genesis in MS is the transient infection or “hit-and-run” hypothesis (1,35). In this scenario, the pathogen is present only briefly in the host, but this is sufficient for a persistent organ-specific autoimmune process to be established (37). The virus acts as a triggering agent only and may be undetectable when clinical symptoms develop. Demyelination could then be induced in several possible ways. As discussed previously, the infectious agent might contain structural sequences identical with a brain protein or other antigen (molecular mimicry). An immune response to the agent then cross-reacts with the corresponding brain antigen, resulting in a chronic, self-perpetuating inflammatory disease. Streptococcus-induced rheumatic heart disease may be an example of this type of autoimmune organ-specific disorder, where an antigenic similarity between bacterial M protein and cardiac myosin leads to cardiac valvular damage (1,14,39). Consistent with this mechanism, many bacterial and viral decapeptides have been identified with amino acid or structural profiles similar to myelin proteins (18). These include hepatitis B virus, EBV, *Escherichia coli*, adenovirus, influenza, measles, and CDV. Using a different approach, Wucherpfennig and
Strominger screened a large number of peptides for degeneracy of amino acid side chains required for major histocompatibility complex (MHC) class II binding and activation of myelin basic protein (MBP)-responsive T-cells (40). A panel of 129 peptides satisfying these criteria was identified, of which herpes simplex virus, EBV, adenovirus type 12, influenza type A, and *Pseudomonas aeruginosa* peptides gave the greatest activation of MBP-specific T-cell clones derived from MS patients. Collectively, these studies support the concept that multiple common infectious agents have the potential for triggering MS by a molecular mimicry mechanism. An alternative possibility for tissue injury might also involve molecular mimicry between infectious agent and host protein, but instead of a myelin protein, a regulatory protein in the immune system or a critical host enzyme might be the target, resulting in altered immune function, disruption of the blood–brain barrier, or interference with myelin metabolism. This could lead to a less direct but equally devastating immunopathology. Additionally, transient CNS inflammation in a genetically susceptible host could also prime the hosts CNS, leading to periodic bystander demyelination when the systemic immune response is activated (37). Another indirect mechanism for myelin injury could be through the effect of exogenous super-antigens. In this scenario, infectious agents can activate T-cells, including auto-reactive T-cells, by interacting directly with the T-cell receptor resulting in a self-perpetuating autoimmune disease even after the agent is eliminated. Lastly, the agent could infect lymphocytes or other immunocompetent cells, altering delicately balanced control mechanisms, and thereby allowing the emergence of aggressive autoimmune T- or B-cell clones (1,14). Measles and CDV are examples of viruses that produce transient profound immunosuppression and neurological illness in which MBP responsive T-cells can be found in peripheral blood (1,9,14,41,42).

If a virus triggers MS, but is no longer present in the host when neurological disease is manifest, it will be extremely difficult to prove causation (1,14). In such a situation, it will be necessary to have persuasive epidemiological and other laboratory as well as clinical evidence linking the virus to MS. However, several problems exist in attempting to use serology to link a virus to MS. If MS is caused by multiple viruses, there may be considerable variability in viral titers geographically and temporally. For example, CMV-induced GBS may occur in epidemics, with few GBS patients demonstrating positive CMV serology in the interepidemic period (43). Second, because MS is a chronic disorder with a variable, often long latent period before onset of neurological symptoms, one would not expect to see a fourfold rise or fall in antibody titers to the agent or a predominantly IgM antibody response, as usually occurs with an acute infectious process (44). Furthermore, if MS is an uncommon complication of a common infection and the agent does not persist, both MS patients and controls may have similar antibody titers to the agent in CSF and serum with no increase in the CSF to serum antibody index (1,14). For example, it may be difficult to conclude whether an individual had early adult infectious mononucleosis or an asymptomatic childhood EBV infection, or paralytic or nonparalytic poliomyelitis, by measuring IgG antibody titers later in adult life. In addition, one would need to show some specificity of the antibody response by demonstrating no similar increase in antibody titers to other viral or nonviral antigens in MS patients. Similarly, antibody titers should not be solely related to an increase in serum or CSF IgG. In contrast, if MS is caused by an agent that does not usually infect humans, it might be easier to demonstrate higher antibody titers to the agent in patients than in normal individuals (1). An example of this type of serological response is found with human rabies. In this type of situation, there should be fewer MS patients with...
low and more with higher antibody titers to the original as compared to controls. Last, the presence of low antibody titers cannot be taken as proof that an individual has not been previously infected by the agent in question, because after many infections, antibody titers fall over time.

**CANDIDATE AGENTS IN MS**

It is conceivable that multiple infectious agents trigger MS. Unfortunately, if MS is caused by multiple agents, it is unlikely that measures will be available in the short term to decrease MS risk (14). Another possibility is that classic MS is primarily caused by a known or as yet unidentified agent that has not yet been firmly linked to MS. In favor of the unitary hypothesis is the distinct worldwide distribution of MS, restricted age-specific incidence, the effect of migration on MS risk, the relatively low concordance rate in identical twins who share a remarkably close childhood environment and reports, albeit controversial, of clustering of MS or changes in incidence in different locales. If only one or a few agents are responsible for triggering MS, disease incidence may be alterable by development and deployment of appropriate vaccines or antibiotics (14).

Several infectious agents currently remain as viable candidate agents because they may be compatible with the unique worldwide distribution of MS, they induce demyelination in humans or animals, agent-specific antibodies are elevated in the serum or CSF of MS patients, or the agent has been identified in MS tissues (1,14). Measles, human coronavirus 226E, EBV, retroviruses, HHV-6, and *Chlamydia pneumoniae* have attracted interest in recent years in terms of known agents that commonly infect humans. Animal viruses that have attracted the most attention are JHM, a mouse coronavirus; Theiler’s murine encephalomyelitis virus, a picomavirus of mice; and CDV, a morbilliform virus of dogs and carnivores.

**Human Infectious Agents**

*Measles Virus*

Measles virus is an RNA morbilliform virus commonly linked to ADEM or post-infectious encephalomyelitis, although this disorder is now less seen in the western world due to the ubiquitous use of measles vaccine. Following postmeasles encephalomyelitis, MBP-reactive T-cells circulate in the peripheral blood, suggesting a possible autoimmune mechanism of tissue injury in this disorder (9,45). However, patients with postmeasles encephalomyelitis rarely go on to develop MS. This is of interest in suggesting that not all viruses causing acute demyelination of the brain trigger MS, even when autoimmune T-cell clones are present, and favors the role of other infectious agents or the influence of specific host factors in MS genesis. From an epidemiological point of view, measles virus infections often occur at a later age in MS patients than in controls, suggesting that a late age of infection may predispose to a different disease phenotype (17,18). A similar phenomenon has been noted to occur with EBV and poliovirus infections (14,23).

Since the original study by Adams and Imagawa (46), most serological studies have shown an increase in measles antibody titers in the serum and CSF of MS patients (14,30). Although absolute titers are only modestly elevated compared with control, the consistency of these findings lends credence to the possible biological
significance of the observation. A difference between MS patients and normal individuals has also been shown in cellular responses to measles virus. Lymphocytes obtained from MS patients may have a specific cytotoxic defect when reacted with measles-infected cell lines (47). This could theoretically lead to a persistent measles infection in MS patients. Consistent with this possibility, measles virus genome has been identified in some MS brain specimens, but most investigators, using a variety of techniques to identify measles genetic material or proteins, have been unable to confirm these observations (48–54). In terms of immunopathogenesis, measles virus decapeptides have amino acid sequences in common with MBP and proteolipid protein (PLP), both components of myelin, suggesting a potential mechanism for molecular mimicry-induced tissue injury (18,53).

Evidence against measles virus as the cause of MS, in addition to the failure to consistently identify the agent in MS tissue, is mainly epidemiological. There is a lack of correlation between measles infections worldwide and the incidence, prevalence, or clustering of MS. For example, MS prevalence is higher in northern United States and Canada than in the South America, although there appears to be no obvious difference in age of measles infection or measles vaccine exposure in these geographically disparate areas. Controversial epidemics of MS or changes in incidence have occurred in the Faroe (54), Orkney (55), and Shetland Islands (56) as well as elsewhere; these appear to bear no relation to the occurrence of measles infections in these locales. More importantly, there appears to be no decrease in the worldwide incidence of MS (13,57,58), despite increasing use of measles vaccines over the past 30 years, and individuals have been identified who have had typical measles infections after onset of MS (59). In contrast, measles vaccination has largely eradicated SSPE in the western world. Thus, measles is unlikely to be an important major primary cause of MS (1).

Coronavirus

Coronaviruses are single-stranded RNA viruses with positive polarity. Two human coronavirus serotypes have been identified, 229E and OC43 (60,61). Although not clearly documented to cause encephalitis or myelitis, these RNA viruses cause approximately 15% to 35% of all upper respiratory infections in humans. However, the potential for human neurotropism does exist, since a receptor for 229E has been demonstrated in brain synaptic membranes, and cultured human neural cells can be infected with this virus (62). In addition, evidence for molecular mimicry between nonstructural proteins of coronavirus 229E and MBP has been demonstrated. The mouse coronavirus JHM can cause demyelination in mice, rats, and primates (52), and both demyelination and mild neurological disease can be adoptively transferred from infected to normal rats (63) using MBP-stimulated donor lymphocytes.

With respect to MS, Tanaka et al. (64), in an unconfirmed study, observed coronavirus-like particles in the perivascular cuff of an MS plaque. In another unconfirmed study, an increase in CSF, but not serum antibody to coronavirus 229E and OC43, was detected in 26% and 41% of MS patients but not in neurological controls (65). In addition, coronavirus 229E viral RNA was identified by Stewart et al. (61) in 4 of 11 MS brain specimens but in none of 11 controls using reverse transcriptase PCR. No OC43 nucleic acid was found in any of these brain specimens. Subsequently, Talbot et al. (62) extended these studies, finding both the 229E and OC43 strains of coronavirus in MS brain samples (36% vs. 4%), but a difference from controls was only found with OC43. Unfortunately, these provocative observations have not yet been confirmed (66).
Confirmation of the presence of viral genome in MS brain tissue, further evidence that coronavirus strains 229E or OC43 cause human neurological disease, and additional demonstrations of higher serum or CSF antibody to 229E and OC43 in MS patients are needed before considering either of these coronaviruses more seriously as a candidate agent in MS causation (1).

Epstein–Barr Virus

EBV is a human herpes lymphotropic DNA virus which causes infectious mononucleosis. EBV has also been implicated in the causation of Burkitt’s lymphoma, nasopharyngeal cancer, and possibly other neoplastic and autoimmune disorders (67). EBV infects over 90% of humans typically after exposure to oral secretions from a previously infected individual (67,68). After infection, EBV remains latent indefinitely in B-lymphocytes; however, viral reactivation occurs spontaneously and is greatly enhanced following lymphoproliferation or immunosuppression (67).

EBV infections in infants or children are usually banal, whereas infections in adolescents and adults often cause infectious mononucleosis (69). EBV frequently occurs at an earlier age in areas where MS is less common and at a later age where MS is more prevalent (68–71). This hygiene hypothesis is consistent with the observation that not only EBV but also polio, measles, and other pathogens can cause a more serious clinical syndrome when acquired later in life, and might even be responsible for initiating autoimmune disorders such as MS (15). Because infectious mononucleosis is usually due to a late infection with EBV, if a late EBV infection is a trigger for MS, one might expect to see more infectious mononucleosis before disease onset in MS patients than in controls (71). In fact, several case–control studies show a higher risk for MS in patients with a history of prior infectious mononucleosis (15,23,71). However, this type of study can be criticized because of potential inaccuracies in the diagnosis of infectious mononucleosis or on the basis of possible recall bias. One way to avoid these biases is to look at individuals diagnosed with infectious mononucleosis, or even better with serologically proven infectious mononucleosis in a large cohort and determine the subsequent risk of developing MS and other autoimmune disorders in these patients compared with controls (71). In this regard, Marrie et al. (72) carried out a population-based, case–control study using the United Kingdom General Practice Research Database (GPRD), which contains medical information on 8% of the entire population (71). There were 225 MS patients (88% definite, 12% probable) in the database who were matched for age, sex, and physician practice with 900 controls for prior infectious mononucleosis up to the age of onset of MS in index patients. Infectious mononucleosis was found to be associated with a greater than fivefold risk for subsequent MS. However, an increased frequency of respiratory tract infection in the five-week, three-month, and one-year period before the onset of MS was also associated with a significant (1.3 to 2.58) risk for subsequent MS. One potential criticism of this study is that the diagnosis of infectious mononucleosis may not have been based solely on positive serology, and other infectious agents can occasionally cause a mononucleosis-like picture (71). This was not an issue in a Swedish study by Lindberg et al. (73) in which 494 heterophile-positive infectious mononucleosis patients were identified in the registry of the Hospital of Infectious Diseases in Göteborg. These patients were compared with patient records at the regional MS registry. Three patients had infectious mononucleosis before onset of MS. On the basis of age-specific prevalence, the expected number of MS cases was 0.81, with a relative risk of 3.7 ($P < 0.05$, 71).
one-tailed) (71). A similar study in Denmark which used the Danish Multiple Sclerosis Registry and a Danish database of 6000 individuals with heterophile-positive infectious mononucleosis also showed a higher than expected rate of MS in patients with prior mononucleosis (P < 0.05) (74). In critiquing these epidemiologic studies, it should be noted that the number of patients with MS after infectious mononucleosis is small, and the statistical significance based on expected rates is marginal (71). Furthermore, similar risks for developing MS have been described in patients with other late childhood infections, including measles, mumps, and rubella, and it is possible that infectious mononucleosis is an important, but perhaps not the sole, infectious disease causing MS or aggravating existing subclinical MS.

The EBV hypothesis is also compatible with reported migration effects on MS risk (18,70,71,75). Individuals migrating from areas where early EBV exposure occurs would presumably be protected from developing MS in their native locale or in their new home if they migrate later in life to an area with a high MS prevalence (71). On the other hand, the next generation would be expected to have the same risk as other individuals residing in the new geographic area. Studies by Dean and Kurtzke on migrants from the West Indies, Africa, or Asia to Great Britain show exactly this phenomenon (76). It is of interest that a similar phenomenon has been shown for type 1 diabetes in Pakistani migrants to Great Britain (15). Of course, this evidence alone cannot prove that a late infection with EBV is the critical event, but it is consistent with that possibility. Similarly, migrants moving from a high- to low-risk area of MS at a young age might acquire EBV at an early age in their new environment and assume the low MS risk of that area, whereas migration at an older age after late exposure to EBV in the land of origin would not change their inherent MS risk (71). One problem with the age-of-exposure hypothesis is that migrants moving from a high-risk to a low-risk area in late childhood should have a higher risk of MS. Further, there is no clear evidence that EBV exposure occurs earlier in southern than northern United States.

Clustering of MS which might also support the EBV-MS hypothesis has been reported (71). For example, an analysis of 381 MS patients in Hordaland, Norway, concluded that patients within the same birth cohort had lived significantly closer to each other between the ages of 13 and 20 years than was found in controls (77). This is an age period when infectious mononucleosis might be expected to occur. A small temporal MS cluster in Denmark with a link to a specific strain of EBV has also been described (78). In addition, temporal data comparing age-specific incidence rates of infectious mononucleosis and MS are also suggestive of a relationship between EBV and MS (76,79). For example, the shape of the curve for age-specific incidence rate for infectious mononucleosis is remarkably similar to that for MS, infectious mononucleosis occurring approximately 10 years before MS (71). EBV as a cause of MS might also explain the relatively low concordance rate of MS in identical twins, because infectious mononucleosis occurs at a later age, when siblings might be apart, and requires more intimate contact than measles or some other aerosol-borne viruses (71).

EBV has been associated with a wide spectrum of diseases of the central and peripheral nervous systems, including aseptic meningitis, encephalitis, psychosis, cranial neuritis, mononeuritis multiplex, brachial plexopathy, GBS, cerebellar ataxia, transverse myelitis, and postinfectious encephalomyelitis (71,80–82). In one study, a primary EBV infection in five patients resulted in a chronic illness indistinguishable from MS (71). This is an important evidence for the EBV-MS hypothesis; however, it remains possible that the infection triggered an MS attack in individuals with
pre-existing, subclinical MS (71). Although EBV frequently causes neurologic disease in humans, there are no data supporting such involvement in other species. This is consistent with the lack of a close animal model of MS.

Serologic studies of serum and CSF have shown an increased frequency of EBV seropositivity, as well as higher titers of antibodies in children and adults with MS (71,83–87). Approximately 99% of adult MS patients are seropositive for EBV, compared with 84% to 95% of controls (88–90). In a recent study of childhood MS (71), 83% of patients, compared with 42% of controls, showed antibodies to EBV ($P < 0.001$), whereas no such difference was seen for herpes simplex, CMV, parvovirus, or varicella zoster (83). Antibodies in MS patients react to multiple EBV antigens, including EBNA-1 and EBV capsid antigen. A similar frequency of positivity to some EBV antigens has been found in patients with systemic lupus erythematosus, rheumatoid arthritis, and Sjogren’s disease, compared with controls leading Pender (91) to postulate that EBV may cause several autoimmune diseases in addition to MS.

In addition to frequency of seropositivity, higher titers of serum antibodies to EBV antigens have been found in MS patients, ranging up to five times higher than that in controls (71). Recently, EBV viral titers have been shown to be elevated even before clinical onset of MS. In the long-term Nurses’ Health Study, 230,000 registered female nurses have been followed since 1976 (92). Of these, 18 women had blood samples collected before their first symptom of MS. On average, these women had higher EBV antibody titers than matched controls ($P < 0.05$). In a similar but larger study of more than 3,000,000 U.S. military personnel, 83 MS patients were identified who had blood drawn for a mean of four years before onset of clinical MS (93). The risk of developing MS increased dramatically with elevation of IgG antibody titers to EBV capsid antigen or EBV nuclear antigen (EBNA). For example, the relative risk of developing MS in patients whose EBV capsid antigen antibody titer was 1:2560 or greater as compared to patients with the lowest level of antibody was 19.7 ($P = 0.004$). Similarly, relative risk for those with the highest level of EBNA antibodies compared with the lowest level was 33.9 ($P < 0.001$). No association was found between CMV antibodies and MS. Unfortunately, one cannot conclude from these studies that these individuals did not already have subclinical MS or that antibody titers to other candidate agents might not be elevated (71).

Support for a possible role of EBV antibody titer as a barometer of MS clinical disease activity was found by Wandinger et al. (94). An association between EBV antibody titer, serum EBV DNA, and clinical course was seen in a longitudinal evaluation of 19 MS patients evaluated monthly for one year. IgM and IgA antibodies to EBV early antigens (P54 and P138), as well as EBV serum DNA, were seen in 72.7% of patients with exacerbations but not in clinically stable patients during this period. In contrast, no relationship between IgG antibodies to EBV and disease activity was found by Myhr et al. although serum EBV DNA was not measured, and a different technique for antibody measurement was used (71,81).

As with serum studies, elevated antibodies to EBV antigens can be found in MS CSF (95,96). In one unconfirmed report, comigration of EBNA-1-specific oligoclonal bands (OCBs) with total IgG OCBs was found in 5 of 15 MS patients and 0 of 12 controls. In this study, an increased CSF antibody index to EBNA-1 was found in the EBNA-1-positive MS subgroup ($P < 0.01$) but also to a lesser extent for the measles virus antibody index ($P = 0.058$). In one patient with CSF EBNA-1 antibody, EBNA-1 was able to absorb a portion of total IgG but not specific OCBs. In another study, CSF from 85% of MS patients contained antibody to EBNA, compared with 13% of controls.
Because of mounting evidence suggesting that EBV might trigger MS and possibly other autoimmune disorders, attempts have been made to identify EBV in brain, CSF, and serum of MS patients using sophisticated molecular techniques. Generally, these attempts have been unsuccessful or nonspecific (97–99). Since the preponderance of evidence indicates that EBV DNA is either not present in MS tissue or present nonspecifically, if EBV is involved in MS pathogenesis it is likely that mechanisms of tissue injury other than a persistent CNS infection are involved. This would support the (hit and run) hypothesis or possibly periodic seeding of the brain by EBV-positive B-lymphocytes. In this regard, evidence of molecular mimicry between EBV and MBP, proteolipid protein, human glial cells, alpha B-crystallin, lymphocyte proteins, and other antigens has been demonstrated and EBV infected lymphocytes could be driven to cause autoimmune disease (71).

In summary, the EBV-MS hypothesis is supported by its compatibility with MS epidemiologic data, the ability of EBV to cause demyelinating disease, EBV-positive serologic studies in MS patients, and the persistence of EBV-infected lymphocytes in human blood. However, despite the growing circumstantial evidence implicating EBV in the causation of MS the case is not proven. Further evidence is needed to conclude whether EBV: causes MS is one of the several agents causing this disease interacts with other pathogens in the causation of MS, or is activated in MS but is unrelated to MS causation. If anti-EBV drugs can ameliorate MS or specific EBV vaccines can be developed with subsequent decrease in MS frequency, critical evidence supporting EBV as a cause of MS would be obtained. Unfortunately, the use of antiviral agents has not yet been shown to influence the course of MS, although it should be noted that antivirals have not as yet been shown to alter the course of infectious mononucleosis either (67,71). In the meantime, EB appears to be the leading human candidate agent for causing MS (71).

Retroviruses

Human retrovirus elements (HERVs) are widely represented across the human genome and probably represent remnants of ancient human retroviruses, which have been incorporated into germline DNA (100,101). HERVs are probably defective, i.e., there is no direct evidence for the synthesis of infectious particles, viral transmission, or synthesis of functional viral proteins (100,101). Nevertheless, a small subset of HERVs possess intact reading frames and therefore have the potential to express retroviral proteins, influence cell function or even through transcomplementation to produce whole virions. HERVs can be subdivided in several ways including the characteristics of their transfer RNA primer, i.e., HERV-W, HERV-K, etc.

The persistence of HERVs in human tissues has led to a search for their functional role in human biology and disease genesis. Although not proved, HERVs have been implicated in both carcinogenesis and autoimmune disorders in animals and humans (100,101). It is not surprising, therefore, that evidence for retroviruses in MS pathogenesis has been sought. For example, HTLV-1, a retrovirus which causes spastic tropical spastic paraparesis was identified in some patients thought to have MS, although further studies could not substantiate a relationship between this virus and MS based on genome identification, serology, or epidemiologic considerations (102). Another human retrovirus (HIV) can cause a chronic neurologic disorder with acute and chronic demyelinating features but has also not been implicated in MS causation.

More recently, additional reports linking HERVs to MS have appeared (103–106). Greenberg et al. (106) found DNA sequences homologous to a human
retrovirus in 6 of 21 MS patients but not in DNA samples from 35 normal individuals. Subsequently, Perron et al. (103,104) isolated a retrovirus (MSRV) now recognized as a member of the HERV-W family in a leptomeningeal cell line obtained from the CSF of MS patients. Similar results were obtained when B-cells from MS peripheral blood or choroid plexus cells from MS brain were cultured (104). MSRV replicated in infected monocytes and both reverse transcriptase activity and a retrovirus-like agent could be demonstrated in culture supernatants. Transcribed MSRV pol gene sequences were detected in the CSF of 5 of 10 MS patients but in none of 10 patients with other neurological diseases and MSRV RNA was also found in the sera of 9 of 17 MS patients but only 3 of 44 controls. Sequencing of MSRV genome showed similarities but also differences with the HERV ERV-9 (107). ERV-9 is found in most human tissues including normal brain white matter. HERVs have also been found in EBV-transformed B-cells obtained from MS patients, raising the possibility that a combination of EBV and HERVs could be cofactors in causing MS (107). However, using a different but ultrasensitive reverse transcription technique, others have been unable to find a similar retrovirus in MS CSF, serum, or peripheral blood mononuclear cells (108).

Serological studies in a few MS patients demonstrated serum and CSF antibodies reactive by western blots with MSRV proteins (3); however, autoantibodies cross-reactive with HERV proteins have been found in patients with other autoimmune diseases, including systemic lupus erythematosus and Sjogren’s syndrome (109), suggesting a lack of serological specificity for MS.

In terms of mechanism of tissue injury, HERV proteins including the HERV-W envelope protein may act as a super-antigen, can influence cellular genes involved in immunoregulation, and may be gliotoxic (101,110). Further, human endogenous retrovirus peptides induced more proliferation and type 1 cytokine production in peripheral blood mononuclear cells from patients with active MS than in patients with stable disease or healthy controls (109).

Although the concept that a unique, endogenous retrovirus could cause MS is attractive, questions have been raised about the specificity of these viruses for MS (111). For example, in a study of Sardinian MS patients, MSRV was found in both CSF and serum of patients and neurologic controls suggesting that MSRV may be a marker of neurologic diseases of inflammatory origin rather than a causative agent (112). Lastly, HERV-W sequences appear to be nonspecifically increased in the brains of patients with inflammatory disorders including HTL and MS, probably driven by brain macrophage activation and increased levels of CNS TNF-α (101).

At present, the role of MSRV and other retroviruses in MS is unknown but because of their ubiquitous nature, more evidence is needed before implicating these agents directly or indirectly in the causation of MS.

**HHV-6**

HHV-6, a recently discovered DNA virus, causes exanthem subitum (roseola) in children. Two variants of HHV-6, A and B have been described. HHV-6B causes most human infections whereas no specific human disorder has been linked to HHV-6A. HHV-6 typically causes rash and fever in children but, in addition, this virus commonly enters the CNS during acute primary infections, occasionally resulting in meningitis or other neurological complications (4,113–115). HHV-6 has also been reported to cause encephalitis in immunosuppressed adults and has been linked in a few instances to encephalopathic and myelopathic disorders as well as to human demyelinating disease (116–122).
Almost all children are infected early in life by this ubiquitous virus, with HHV-6 seropositivity being seen in 90% of all children by two years of age (113–115). Like other herpes family viruses, HHV-6 persists lifelong in brain and other tissues in most normal individuals, and—as with other herpes viruses—HHV-6 may be reactivated nonspecifically.

Interest in HHV-6 as a candidate agent in MS intensified following the report by Challoner et al. (4) in 1995, demonstrating HHV-6 variant B group 2 in the brains of greater than 70% of patients with this disease. Although HHV-6 was found in a similar percentage of control brains, viral proteins were identified by immunocytochemistry in oligodendrocytes from 12 of 15 MS brain samples but in none of 45 control brains (4). These proteins were preferentially expressed in MS plaques rather than in histologically uninvolved white matter. Similar immunocytochemical findings were found by Knox et al. (123) who reported that 17 of 19 tissue sections that were undergoing active demyelination, obtained from six MS patients, contained HHV-6 proteins, versus none of 15 brain samples from patients with other inflammatory demyelinating diseases. In addition, Knox et al. found HHV-6 antigens in six of nine MS lymph nodes but not in lymphoid tissue from seven controls.

In contrast, other investigators have not identified HHV-6 in any tissue from MS patients (124–126) while some investigators, although finding HHV-6 genome or antigen in MS brain, have noted the lack of specificity for either MS CNS, brain cell types, or to areas of demyelination (127,128). Because herpes viruses can be activated nonspecifically by trauma or alteration in immune status, it is conceivable that the intense inflammatory response, along with the cell death and proliferation that occurs in MS lesions (129), reactivates latent HHV-6. Similarly, HHV-6 could be nonspecifically reactivated in patients previously immunosuppressed with steroids or other drugs. This might explain why brain material from patients with SSPE, progressive multifocal leukoencephalopathy, and HIV can show similar HHV-6 brain findings as in MS (127,128).

HHV-6 variant A and B DNA have also been identified in blood or CSF from some MS patients (123,130–141), but again others have not confirmed these findings or find them to be nonspecific (113,130,133–135).

As to serological responses, reports of higher IgG or IgM antibody titers to HHV-6 or HHV-6B in serum or CSF of MS patients versus controls have been found in several (113,114,131,132,136) but not all studies (137–140) and elevated titers may also be found to other viruses and in other disorders. Similarly, the lymphoproliferative response to HHV-6 antigens as compared to controls has been conflicting (141,142).

Some have reported a relationship between MS disease stage or activity and the presence of HHV-6 DNA, mRNA in blood, or serum IgM antibodies to HHV-6. For example, Villoslada et al. found increased anti HHV-6 IgM serum antibodies in patients in the early phases of MS including patients with a clinically isolated syndrome as compared to patients with secondary progressive MS. This response was not specific since in the same patients IgG antibodies were elevated to EBV and two patients with other neurological diseases also had elevated IgM antibodies to HHV-6 (144). In another study, serum HHV-6 DNA was found in significantly more patients experiencing a clinical exacerbation of MS (4 of 18 samples) as compared to patients deemed to be in clinical remission (11 of 197 samples; P = 0.008) (143). In the most recent study by Alvarez-Lafuente et al. (144) evidence of an active HHV-infection as indicated by HHV-6 DNA in serum and HHV-6 RNA in blood was detected in 16% of patients with RRMS but in no healthy controls (P = 0.003). Among those RRMS patients with active viral replication, viral load was higher during an acute attack than in remission.
(\(P = 0.04\)). Interestingly, only the HHV-6 A variant was detected, suggesting this variant might be more specifically related to MS.

Although it is difficult to reconcile the pattern of early HHV-6 infection with the worldwide pattern of MS, twin studies, and migration effects, the ultimate test of the HHV-6 hypothesis awaits additional studies and attempts at modifying disease course with antiviral drugs. With regard to the latter, no significant clinical benefit of acyclovir given in a dose of 2.4 g daily was seen (145). Because of concerns about adequacy of dosage and spectrum of the antiviral effect of acyclovir, another study was carried out with valacyclovir, a prodrug of acyclovir that increases acyclovir bioavailability severalfold (146). Again, no significant difference in MRI activity or clinical relapses was found in this double-blind placebo-controlled randomized trial. These therapeutic trials do not exclude the possibility that HHV-6 contributes to MS lesion pathogenesis because sensitivity of the virus to the drug or drug bioavailability may be insufficient to benefit MS in the short term. However, weighing all the evidence to date, it seems more likely that HHV-6 is a passenger rather than the driver in MS causation.

Chlamydia pneumoniae

C. pneumoniae, an obligate intracellular bacterium closely related to other chlamydial species including Chlamydia psittaci, Chlamydia trachomatis, and Chlamydia pecorum, was first described in 1986 by Grayston et al. (147). Seroepidemiological studies indicate that about 80% of the population has been exposed to this organism, usually in childhood and young adult life (148,149).

Chlamydial infections are typically mild or asymptomatic but because they may go unrecognized they can cause a chronic low-grade infection. C. pneumoniae is thought to be responsible for approximately 10% of community-acquired pneumonias; other acute symptoms such as headache, abdominal complaints, pharyngitis, and bronchitis are common (148–150). Chlamydia species may also cause neurological disease. For example, C. pecorum has been implicated as a possible causative agent in sporadic bovine encephalomyelitis, and a variety of neurological disorders including meningoencephalitis and GBS have been described with other chlamydial species, including C. pneumoniae. Natural infection with Chlamydia does not necessarily confer lasting immunity, so that reinfections can and do occur. In addition, following immunization against C. trachomatis, reinfections can be clinically more severe than in a primary infection (148).

Several lines of evidence suggest that C. pneumoniae may cause or contribute to atherosclerosis (149,150). For example, seroepidemiological studies suggest that the presence of antibodies to C. pneumoniae doubles one’s risk for heart disease. In addition, C. pneumoniae has been found to be present in atherosclerotic lesions by a variety of techniques including PCR, immunocytochemistry, in situ hybridization, enzyme-linked immunosorbent assay (ELISA), and electron microscopy. C. pneumoniae has also been cultured from atherosclerotic arteries. These provocative studies have led to ongoing multicenter trials to determine if treatment with appropriate antibiotics alters the natural history of atherosclerotic complications.

Two chronic neurological disorders have been associated with C. pneumoniae. Balin et al. (151) using PCR identified C. pneumoniae DNA sequences in the brain lesions of 17 of 19 patients with late-onset Alzheimer’s disease (AD) but in only 1 of 19 controls. Electron microscopy, immunoelectronmicroscopy, reverse transcriptase PCR assays, and immunohistochemical studies also identified C. pneumoniae antigens, transcripts, or C. pneumoniae-like organisms in AD brain specimens, the
latter being successfully cultured from AD but not control brains. The demonstration of C. pneumoniae in AD brains by multiple techniques has lent credence to the observation. Unfortunately, at least two other groups using immunocytochemistry and PCR techniques have failed to confirm the findings of Balin et al. (152,153). While technical differences could explain the difference in results, enthusiasm for an AD-Chlamydia link has waned.

In 1999, Sriram called attention to a possible link between C. pneumoniae and MS (154). In their initial patient with rapidly progressive MS, C. pneumoniae was isolated from the CSF, and treatment with antibiotics resulted in marked neurological improvement. In a follow-up study of 37 patients with MS (17 relapsing-remitting, 20 progressive) and 27 patients with other neurological diseases, C. pneumoniae was isolated from the CSF of 64% of MS patients versus 11% of controls (5). By PCR, C. pneumoniae MOMP gene was identified in the CSF of 97% of MS patients as compared to 18% of controls; by ELISA, 86% of MS patients had C. pneumoniae antibody levels three standard deviations greater than those of controls. The specificity of the antibody response was confirmed by western blot assays following isoelectric focusing of MS CSF. These assays revealed the presence of cationic antibodies in MS CSF reactive against several C. pneumoniae elementary body antigens, particularly to a 75-kDa protein. Sriram also reported that OCBs in MS CSF were partially or completely adsorbed following exposure to C. pneumoniae antigens but not to viral or neural antigens, whereas OCBs in CSF from patients with SSPE were adsorbed by measles but not C. pneumoniae antigens (155). As yet, no reports have confirmed Sriram’s observation that MS CSF OCBs, but not control OCBs, react specifically with C. pneumoniae.

In a recent prospective study, Munger et al. (156) measured IgG and IgM antibodies to C. pneumonia in sera collected from patients prior to the clinical development of MS and closely matched controls. The authors concluded that neither C. pneumonia seropositivity nor IgG antibody titers predicted risk of developing MS; however, because of differences in results between cohorts, they could not exclude the possibility that infection with C. pneumonia might modify risk of MS.

If confirmed, these provocative findings would suggest several possible roles for C. pneumoniae in MS. C. pneumoniae could cause MS, contribute to lesion pathogenesis due to entry of infected macrophages and monocytes into the CNS, or be an infectious bystander of no particular relevance to MS lesion genesis or clinical prognosis. Some support for this controversial hypothesis has come from conflicting reports on the detection of C. pneumoniae in MS CSF (157) and by the demonstration of increased anti-chlamydia IgG among women with MS as compared to controls (158). Unfortunately, several PCR studies have failed to identify C. pneumoniae in CSF, serum, peripheral blood mononuclear cells, or brain samples obtained from a large number of MS patients (159,160). In one of these studies, no increase in the ratio of CSF to serum antibody titers was found to suggest local production of C. pneumoniae antibody within the CNS (160). The possibility that technical differences between studies might have led to false-negative conclusions cannot be excluded; nevertheless, until further evidence is forthcoming, these findings cast doubt on the Chlamydia MS hypothesis.

Animal Infectious Agents

Several animal viruses cause demyelination in their natural hosts. These include visna, Theiler’s virus, murine coronavirus, and CDV. Even if not causative for MS, these animal models may provide valuable insight into mechanisms of virally induced demyelination.
Visna, an RNA lentivirus of sheep, seems unlikely to cause MS, since the worldwide pattern of MS is not compatible with a disease of sheep, and no reports of visna virus genome in MS brain or serological evidence for visna infections of humans have been forthcoming (1). Similarly, antibody titers to the mouse RNA viruses, mouse coronavirus, and Theiler’s virus have not been increased in the serum or CSF of MS patients (1), and Theiler’s virus genome has not yet been identified in MS brain samples. Although a murine-related coronavirus genome was identified in 12 of 22 MS brain specimens by in situ hybridization, and coronavirus antigen was identified in brain material using immunocytochemical techniques from two patients with rapidly progressive MS (52), others have not been able to confirm the presence of mouse coronavirus genome or to isolate murine coronaviruses from MS tissues (1, Dowling, personal communication). Thus, there is little in the way of hard epidemiological, serological, or microbiological evidence to indicate that these animal viruses are likely to cause MS.

CDV

CDV, a single-stranded RNA paramyxovirus of antimessage (negative) polarity, is a member of the Morbillivirus genus, which also includes measles, rinderpest, and the recently discovered seal plague (phocine) virus (1,14). These viruses are of great interest because they are highly contagious in their respective natural hosts (161); can be very neurotropic causing CNS inflammation or demyelination in many species, including humans (1,14,162,163); and can jump species (1,14,160,164,165). A recent outbreak of a fatal disease in horses was thought to be caused by a new member of the Morbillivirus group. This virus was also apparently transmitted from horse to humans, causing a severe respiratory illness in two humans and death in one (166).

CDV infects dogs and other carnivores, including Japanese macaques. Susceptibility extends to a wide range of nondomestic animals and, more recently, CDV has been shown to produce disease in large cats, including lions, tigers, and leopards.

CDV can cause a subclinical disease in dogs but typically results in a febrile illness, with upper respiratory and gastrointestinal manifestations (161). Neurological sequelae are common either in close proximity to infection or after a variable latent period. Animals may develop optic neuritis, myelopathy, or encephalopathy. The neurological illness is commonly acute and monophasic but can be relapsing or progressive (1,14). In the latter situation, the virus can be readily identified in brain tissue, whereas in the latter situation, viral identification can be problematic (167). Some strains of CDV can cause demyelination in up to 90% of dogs, which makes it far more neurotropic in its natural host than measles is in humans (1,14,163). Interestingly, vaccinating dogs with measles vaccine can prevent these neurologic complications. Pathologically, CDV can cause a panencephalitis or primary demyelination, occasionally with plaque-like lesions in periventricular white matter that are difficult to distinguish from MS (1,14,167).

The CDV-MS hypothesis implies that MS should be more common in geographic areas where genetically susceptible individuals have the greatest exposure to dogs (i.e., in areas where dog–human contact is closest and where CDV is common in the canine population) (1,14). Conversely, risk for MS would be expected to be diminished in areas where dogs are uncommon, where dog–human contact is low because of cultural attitudes toward dogs or because dogs are kept outdoors, and in isolated regions where distemper is not endemic (1,14). In this regard, both MS and
dog density (and the indoor dog location for pet dogs) are higher in North America and Europe than in India (168) and, probably, in China and Japan (1,14). Moreover, dogs are more likely to be kept indoors in colder climates, such as the northern United States, compared with the American South (169); dogs are more likely to have epidemics of overt CDV infection in cold, damp climates (DiGiacomo, personal communication); and CDV may survive longer at colder temperatures (170), conditions conducive to greater human-CDV-infected dog contact in regions of greatest MS prevalence (1,14). Examples of a geographic gradient for a dog-linked human infectious disease exist, with human hydatidosis being 10 times more common in colder regions of Kenya, where dogs are kept indoors, than in warmer regions of this country (1,14,171).

If MS is a zoonosis, spread by CDV from dog to human, one would expect MS patients to have more dog exposure before onset of the disease than matched controls. However, this might not be true for individual patients, because CDV, like measles, is an extremely contagious disease, typically spread by a respiratory route and even brief exposure to an infected dog could be sufficient to cause infection (1,14). The problem with epidemiological studies of dog exposure is the high background noise, as 60% to 80% of controls in some American and European studies own dogs, indicating the need for large numbers of MS patients and controls to properly study this relationship (1,14).

Although most studies of MS patients (involving relatively few individuals) have not shown more dog ownership, dog exposure before onset, or expected onset of MS, at least 11 studies have shown such a temporal (171–181) correlation. However, if CDV is the agent and the dog the vector, then the more important relation is the contact between humans and dogs with distemper and the subsequent development of MS. Three reports of increased exposure of MS patients to dogs with a CDV-like illness before onset of MS have been published (172,182,183), in one of which exposure to dogs with a neurological illness was greater in MS patients than controls in the five years before onset of MS (176). Other studies, although not statistically significant, have shown a trend in this direction (184–186). Of course, there is no documentation that these dogs truly had a CDV infection, and the possibility of recall bias cannot be excluded. Since the availability of distemper vaccine over the past 40 years, overt distemper is now less common than in the past. However, CDV infection still occurs as isolated cases, and occasionally as epidemics even in dogs previously vaccinated with distemper vaccine suggesting that protection from vaccine is not life long, and wild animal vectors as a possible source for CDV infection remain.

Until recently, it was difficult to determine by serological methods whether a human had been infected by CDV because of the similar peptide homologies and antigenic relation between measles virus and CDV (187,188). Several early studies searching for serum antibodies to CDV showed higher titers in MS patients than in controls using a tissue culture neutralization assay (1,14,189). In one such study, the highest antibody titers in MS patients were to virulent rather than vaccine strains of CDV, and no significant increase in antibody titer was found to six other dog viruses (190). Smaller studies or those utilizing different techniques found no difference in serum CDV titers between patients and controls (1). Unfortunately, these serological studies were unable to distinguish definitively between CDV antibody and cross-reacting MV antibodies.

In 1995, following the publication of the entire nucleotide structure of CDV and measles, Rohowsky-Kochan et al. (191) were able to select peptide sequences present
in the surface CDV hemagglutinin H protein, which had predicted antigenic determinants that differed structurally from corresponding measles peptides (1,14). They synthesized three such CDV H peptides, each 15 to 16 amino acids in length, which—in addition to being structurally different from measles virus—were also structurally different from each other (1,14,158). In studies of animals and humans vaccinated or infected with the measles virus and with high measles antibodies titers, the discriminatory capacity of the assay was demonstrated. None of the measles antibody-positive sera reacted with CDV in ELISA, whereas animals immunized with CDV reacted with all three CDV peptides (1,14,191). Subsequently, in a survey of large numbers of MS patients, age-sex-matched normal individuals and patients with other neurological and inflammatory diseases, a significant increase in serum CDV antibody titer to all three peptides was found only in the MS patients (1,14,191), with titers being significantly elevated over a wide age span (192). Some 70% of all high-titered CDV sera belonged to MS patients, indicating a relatively high degree of specificity, although not sensitivity, for this assay. A striking relationship was also observed between elevated CDV-H antibody levels and the diagnosis of MS ($P < 0.0001$, odds ratio $= 5.0$) (163). In contrast, no increase in viral antibody titer was found to varicella zoster or polio virus in these studies nor was there a relationship between CDV titer and serum IgG levels (1,14,191,192). These results suggest that humans can be infected by CDV, and are consistent with, but do not prove the hypothesis that MS may in some instances be triggered by this agent (1,14,191,192).

The criticisms of the CDV-MS hypothesis include the failure to date to find CDV protein or genome in MS brain (51,192,193), the high titers of CDV antibody that can occur in some individuals without MS, the low titers of CDV antibody in many patients with MS, lack of studies to show whether CSF OCBs bind to CDV, and the failure of MS to decline since the availability of distemper vaccine.

In summary, the possibility that MS is a zoonosis remains viable and canine distemper remains a leading candidate agent for triggering MS in some patients. However, more studies are needed to link CDV to MS.

CONCLUSION

In summary, we have reviewed the evidence favoring an infectious cause of MS. Possible mechanisms for infection-induced demyelination has been described. Epidemiological, serological, and other data in support of several human and animal candidate viruses have been presented. No single agent has yet been unequivocally linked to MS. Recommendations for further research are provided, but—as has been pointed out previously by Bernard and Simini—sublata causa tollitur effectus (194): a causal link can be invoked only after removal of the hypothetical cause has been shown to eliminate the effect.

REFERENCES


Multiple Sclerosis: An Autoimmune Disease of the Central Nervous System?

John R. Rinker II, Robert T. Naismith, and Anne H. Cross
Department of Neurology and Neurosurgery, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

INTRODUCTION

Multiple sclerosis (MS) has long been accepted as an inflammatory disease limited to the central nervous system (CNS). The etiology of the disease, particularly the initial inciting event, remains unknown. The purpose of this chapter is to outline the evidence supporting an autoimmune etiology for MS. In presenting this evidence, it is helpful to review the revised postulates of Witebsky, published by Rose and Bona in 1993, which establish the criteria for denoting a disease as autoimmune in origin (1). The original postulates required that an autoimmune response be recognized in the form of an autoantibody or cell-mediated immunity; the corresponding autoantigen be identified; an analogous autoimmune response be induced in an experimental animal, and the immunized animal then develop a similar disease. The revised postulates sought to distinguish pathogenic from nonpathogenic B- and T-cell responses and to characterize the evidence for these responses into direct proof, indirect evidence, and circumstantial evidence.

Firm direct evidence for MS as an autoimmune disease is lacking. On the basis of similarities to the animal model experimental autoimmune encephalomyelitis (EAE) (discussed later in this chapter), MS is thought to be mediated, at least in part, by T-lymphocytes. Ethical considerations, as well as major histocompatibility complex (MHC) incompatibility, preclude experimental human-to-human or human-to-animal cell transfer, which might prove the idea that MS is autoimmune and transferred by immune cells or humoral factors directed against nervous system constituents. However, indirect evidence of autoimmunity in MS is abundant, and this chapter outlines the available evidence that MS is an autoimmune disease.
EPIDEMIOLOGY

Autoimmune diseases are often associated with a higher-than-expected incidence of other autoimmune diseases within the same patient and among family members (2). For example, myasthenia gravis (MG), a well-defined antibody-mediated autoimmune disease, is associated with co-present autoimmune diseases at a rate higher than expected for the general population (3). Multiple investigations have sought an association of MS with other autoimmune diseases, but to date there is little evidence of a disproportionate co-occurrence of other autoimmune diseases with MS. In a retrospective series of 826 MS patients, a collection of autoimmune diseases was identified (15 hyperthyroidism, 4 primary myxedema, 5 rheumatoid arthritis, 4 type 1 diabetes mellitus, 2 ulcerative colitis, 2 vitiligo, and single cases of other diseases). The cumulative prevalence of associated autoimmune disease in this cohort was 4.9%, no higher than expected for the general population (4). Several smaller studies have found higher than expected rates of other autoimmune diseases in patients with MS. One study found a threefold increased incidence of autoimmune thyroid disease among 188 MS patients (5), and at least two studies have reported an increased association of MS with MG (6,7).

PATHOLOGY SUGGESTS AN AUTOIMMUNE ETIOLOGY

MS tissue pathology suggests an immune-driven reaction to a myelin antigen. The pathology indicates a complex disorder that involves all arms of the immune system including cellular immunity, humoral immunity, and complement (8,9). Structures of the nervous system other than myelin, such as the axon and the oligodendrocyte cell body, may be lost or injured, thus making it difficult to identify the initial target. Even at very early stages, MS lesions demonstrate axonal as well as myelin damage (10). The pathology in MS appears to be heterogeneous (9), signifying variation in the innate immune response(s) and/or the inciting event(s).

Active MS lesions are characterized by immune cell infiltration, predominantly by T-cells and macrophages (11), as well as the presence of immune mediators such as adhesion molecules, chemokines, cytokines, and matrix metalloproteinases (MMPs). CNS vascular endothelium from MS patients expresses surface antigens such as ICAM-1, VCAM-1, E-selectin, and MHC II, all of which facilitate leukocyte adhesion and migration from the peripheral blood into the CNS (12). MHC II-associated antigens, essential for T-cell activation, have also been demonstrated on astrocytes within acute, chronic, and silent plaques (13–15). MMPs are enzymes that contribute to the breakdown of the extracellular matrix, facilitating trafficking of immune cells through the neuropil (16). MMPs may also be directly toxic to CNS structures (17). Histologic studies have noted that endothelial cells in MS lesions express MMP-3 and -9. Messenger RNA expression for MMP-7 and -9 is upregulated throughout the brain of MS patients (18). Macrophages in active lesions express MMP-1, -2, -3, -7, -9, and -12 (19–21). Studies of serum and CSF show increased expression of MMP-9 (22–25) during MS disease activity.

Many different chemokines, including IP-10 and Mig, have been found within the MS plaque. Some macrophages and CD3+ T-cells within MS-affected CNS express the CXCR3 receptor for these chemokines (26,27). CXCR3 positive T-cells are increased in CSF during relapses (28,29). CCR1 and CCR5 positive cells have been found in some MS lesions (30). One study suggested that primary progressive
MS patients have higher CCR5 mRNA expression by peripheral blood mononuclear cells than other forms of MS, but the surface protein expression of CCR5 on CD4+ T-cells from PBMC was similar in all MS subtypes and in controls (31). Both “pro-” inflammatory (IL-2, IFNγ, TNFα) and “anti-” inflammatory (TGF-β, IL-4, IL-10) cytokines are expressed by infiltrating cells, astrocytes, and microglia within MS lesions (32–35). The presence of both Th1 and Th2 cytokines in active and chronic lesions suggest a finely orchestrated response of the immune system to some unknown stimulus. Activated macrophages are a prominent component of the MS lesion (36,37), and can be seen adjacent to the axon apparently stripping it of myelin (38,39). Antibodies and complement that coat myelin in some MS lesions have been implicated as opsonins in this phagocytic process (38,40).

Although MS pathobiology does not prove an autoimmune cause of MS, it confirms that the immune system plays a central role in the disease process. It also reveals that immune system activity continues for decades in the absence of a recognizable pathogen. The animal model, EAE, which is autoimmune and initiated by T-cells, mimics MS in many aspects of the gross and microscopic pathology.

GENETIC EVIDENCE SUPPORTING AUTOIMMUNITY IN MS

Epidemiologic data strongly support a genetic predisposition to MS. Other putative autoimmune diseases such as SLE and rheumatoid arthritis are well documented to occur at a higher frequency within families of affected patients than in the general population (41). Twenty percent of patients with MS report a positive family history for MS (42,43). Approximately 30% of monozygotic twins are concordant when one of the twins has MS, while among other siblings the risk is 2% to 5%, and among half-siblings the risk is 1.1% to 1.4% (44). Among adopted siblings and the general U.S. and Canadian populations, the risk is 0.1% (45,46). The familial predisposition to MS has prompted investigations into immune-response genes to explain this enhanced susceptibility.

MHC-II molecules are expressed on the surface of antigen-presenting cells and present processed antigen to CD4+ T-cells (47). The CD4+ T-cell, in turn, is the primary mediator of the animal model for MS, EAE (48), and is strongly implicated in MS. A number of purported autoimmune diseases including rheumatoid arthritis (49), type 1 diabetes mellitus (50), and MS (51) are associated with specific MHC-II haplotypes. This suggests immune mechanisms in their pathophysiology. The MHC II DR2 (HLA DRB1*1501 and DRB1*1503) haplotype has been found to convey susceptibility to MS (51,52). In addition, a “dose effect” of HLA-DR2 haplotypes on both susceptibility and progression of MS has been documented. Patients with two copies of HLA-DR2 have an increased risk of developing MS and of having a more severe course compared with heterozygotes (53). Different HLA II genes appear to influence disease susceptibility in people of non-European descent (54).

Investigations of other immune system-related genes have yielded data in support of an environmental effect superimposed on the genetic predisposition for the immune dysfunction. Utz et al. examined T-cell receptor (TCR) gene usage in T-cells reactive with myelin basic protein (MBP) and tetanus toxoid from concordant and discordant monozygotic twins. They found that MS-affected twins’ T-cells selected Vβ8 TCR after stimulation with MBP, whereas nonaffected discordant twins selected different TCRs (55). These and earlier studies (56) implicate the T-cell
as a contributor to MS pathogenesis. In a follow-up study of five pairs of monozygotic twins (two discordant sets, two concordant sets, and one healthy set), Utz et al. (57) confirmed the over expression of Vα8 in MBP-specific cells from MS patients, and examined the complementarity-determining region 3 (CDR3) of Vα8-positive TCRs. The latter studies demonstrated a profound heterogeneity of CDR3 usage, which correlated with disease severity. The extensive heterogeneity was restricted to MS-affected subjects, and was limited to T-cells specific for MBP and not seen in cells specific for tetanus toxoid. The data were interpreted as being suggestive of a role for MBP-reactive T-cells in MS pathogenesis.

The theory of autoimmune disease postulates that loss of T-cell tolerance to self-antigens underlies the development of the autoimmune reaction. Dysfunction of the CTLA-4 receptor is one possible mediator of this lack of self-tolerance. Both CD28 and CTLA-4 are T-cell receptors for the costimulatory B7 molecules expressed on antigen presenting cells (APC). Most T-cells bear the CD28 receptor, which upon ligation contributes to T-cell activation with ensuing secretion of the pro-inflammatory cytokine IL-2 (58). T-cell activation induces expression of CTLA-4 (59). Ligation of CTLA-4 by B7 molecules, on the other hand, causes T-cell inactivation. Oliveira et al. (60) sought to determine whether the CTLA-4 receptor behaved differently in MS patients compared to controls. Blocking the CTLA-4:B7 interaction, following stimulation with MBP, led to increased proliferation and cytokine production by T-cells from healthy controls compared with MS patients. Thus, MS patient cells may have impaired sensitivity to the regulatory effects of CTLA-4. Polymorphisms of the CTLA-4 exon 1 have been associated with rheumatoid arthritis, Grave’s disease, type 1 diabetes mellitus, and MS (61,62). Presence of the A allele of the CTLA-4 gene may convey a worse prognosis with regards to MS progression (63).

EVIDENCE FOR T-CELL MEDIATED AUTOIMMUNITY IN MS

Interest in a potential autoimmune explanation for MS led to a search for T-cells reactive to myelin antigens. These antigens include MBP, myelin associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP). Increased frequencies of PLP-reactive T-cells are reported in both blood and CSF of MS patients compared to controls (64,65). However, it has been shown that healthy controls harbor T-cells in their peripheral blood also reactive to MBP, MAG, and MOG in frequencies similar to MS patients (66). Thus, investigators have sought to demonstrate differences between MS patients and controls in the fine specificity, functional state, and activation state of these myelin-reactive T-cells.

The fine specificity of a TCR denotes its specific recognition of an antigen epitope. MBP83–99 has been cited as the human immunodominant sequence within MBP (67), but T-cells reactive to this epitope have been identified in healthy controls as well as MS patients (68). Likewise, investigation into the fine specificity for T-cell recognition of MOG has yielded no clear distinction between MS patients and healthy controls, including recognition of the immunodominant regions of MOG (a.a. 11–30) (69). This same region has proven encephalitogenic in the EAE model (70). Pelfrey et al. (71) found that T-cells from MS patients and healthy controls responded to many different epitopes of PLP, scattered throughout the molecule. However, MS patients responded to four times more peptide sequences of PLP than controls, and they had 11 times higher numbers of PLP peptide-specific IFN-γ-producing cells than controls.
T-cells from MS patients and controls also differ in cytokine-secreting profile upon activation. MHC II-restricted CD4+ T-cells that manufacture IFN-γ, IL-2, lymphotixin, and TNF-α are defined as Th1 cells and may be thought of as “pro-inflammatory” cells promoting disease in MS. Functions of Th1 cytokines include immune cell activation and induction of adhesion molecule expression, recruitment of additional immune cells, and perhaps direct mediation of myelin damage. T-cells producing IL-4, IL-5, IL-10, and IL-13 are termed Th2 cells and promote antibody-mediated, immune complex, and allergic disorders. In the context of MS, these cells are considered “anti-inflammatory” and antagonistic to the effects of Th1 cells (72,73).

In reality, human T-cells do not strictly conform to the dichotomous cytokine expression patterns of Th1 and Th2-cells as seen in mice and it is an oversimplification to consider these as “pro-” and “anti-inflammatory,” respectively. Some studies have suggested a tendency for myelin-reactive T-cells in MS patients toward the Th1 phenotype. For example, Correale et al. (74) found that T-cell clones to PLP generated during acute MS attacks were skewed toward Th1 phenotypes. During disease quiescence, clones showed Th0, Th1, and Th2 phenotypes. Several investigators have reported increased expression of the chemokine receptor CCR5, characteristic of Th1 cells, and its corresponding chemokines in the CSF and CNS tissues from MS patients (75–77).

Hellings et al. (78) demonstrated a temporal association between clinical disease activity and antimyelin T-cell responses. Earlier studies of a limited number of MS patients also suggested such an association (79). Soderstrom et al. (80) observed increased levels of T-cells recognizing MBP, PLP, and myelin associated glycoprotein in peripheral blood and CSF of untreated MS patients, but did not observe an association of T-cell responses with disease activity. Hellings found a number of immune changes coincident in some instances with the detection of active lesions by MRI or with clinical exacerbations. These changes included an increase in myelin-reactive IFN-γ secreting T-cells, clonally expanded myelin-reactive T-cells, elevated pro-inflammatory and decreased anti-inflammatory cytokine production, upregulation of ICAM-1, and highly increased serum soluble VCAM-1.

Clearly, the mere presence of myelin-reactive T-cells in the periphery is not sufficient to cause MS. It has been reasoned that if myelin specific T-cells caused MS, these cells would show signs of prior activation. Several different lines of investigation have shown that in many MS patients myelin reactive T-cells have been previously activated. Zhang et al. examined whether peripheral blood-derived myelin-reactive T-cells in MS patients existed in a different state of activation compared with healthy controls. Activated T-cells, but not resting T-cells, express IL-2 receptors. In an in vitro study, no difference in the frequency of MBP or PLP-reactive CD4+ T-cells was found after primary antigen stimulation between RRMS patients and normal controls. However, when cells were first cultured with recombinant IL-2 to enrich IL-2 receptor positive cells prior to stimulation with antigen, the frequency of MBP and PLP-reactive T-cells was higher in MS patient cell lines than in controls. In CSF samples, MBP-reactive T-cells were recovered from MS patients but not from controls. In the CSF, IL-2 stimulation yielded MBP-reactive cells more than 10-fold higher in paired blood samples (81) indicating that these activated MBP-specific T-cells entered the CNS.

T-cells that have been activated previously do not require B7 costimulation of CD28 for reactivation. Thus, another method of demonstrating prior activation of myelin-reactive T-cells is to quantify the number that do not require costimulation for activation. Using cell transfectants expressing MHC-II DR2 alone or cotransfected
with human B7-1 or -2, to present the immunodominant MBP_{85-99} to purified CD4^{+} T-cells from DR2^{+} RRMS patients and controls. Scholz et al. (82) observed that cells from control subjects did not expand in response to the MBP_{85-99} in the absence of costimulation, but MBP-reactive T-cells from MS patients were activated without B7 costimulation. Lovett-Racke et al. (83) had a similar rationale when using anti-CD28 antibodies to block costimulation by B7 molecules. In their studies, MBP-reactive T-cell expansion was inhibited by blockade of the CD28:B7 interaction in normal individuals but not in MS patients.

Another marker of previous activation and proliferation in T-cells is genetic mutation. T-cells that have proliferated previously can develop mutations in the hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene; these cells may then be isolated by exposure to 6-thioguanine, which is toxic to nonmutated cells. Allegretta et al. (84) identified MBP and MBP-peptide specific HPRT mutant T-cells from the peripheral blood of MS patients but not from controls. Later experiments did find HPRT mutants in control blood, but to a lesser degree than in MS patients (85,86). Trotter et al. (87) found significantly more HPRT mutant T-cell lines in MS patients than controls, and in addition some of these mutated T-cell lines recognized multiple epitopes of PLP. During a clinical exacerbation, HPRT-mutant lines derived from one MS patient recognized the specific PLP_{178-191} peptide. These PLP_{178-191} reactive mutant T-cell lines were not detected during remission.

Wulff et al. (88) took another approach to explore the previous exposure of T-cells to myelin antigens as indirect evidence for autoimmune pathogenesis of MS. They exploited their finding that human effector memory T-cells express high levels of the voltage-gated Kv1.3 channel, whereas naive and central memory T-cells express far lower levels. T-cells reactive with MOG, MBP, or PLP from MS patients expressed far more Kv1.3 channels per cell than T-cells reactive with these antigens from control subjects. In contrast, the level of Kv1.3 channels in GAD65-reactive T-cells, insulin-reactive T-cells, and the vast majority of ovalbumin-reactive T-cells derived from MS patients was low and not higher than that for controls. Mitogen-reactive T-cells from MS patients and controls had similar levels of Kv1.3 channels per cell, suggesting that the general level of effector memory T-cells in MS patients was similar to that of the controls. Taken together, data from the studies discussed in the preceding paragraphs strongly indicate that MS patients harbor more previously activated memory T-cells directed against myelin antigens than do control subjects.

Despite the varied studies, indicating that T-cells reactive with myelin antigens are more frequently activated or previously activated in MS patients than controls, it should be recognized that T-cell activation may be secondary to the liberation of myelin antigens that occurs with myelin damage. One manner in which MS might be proven to be autoimmune would entail specific deletion of myelin-directed T-cells in MS patients, followed by sustained demonstration of disease remission. These cell populations have been selectively deleted in vivo by vaccination with autologous myelin-reactive T-cells harvested from CSF, but to date no blinded results demonstrating clinical efficacy have been published (89,90).

Strong evidence that the pathogenesis of MS is autoimmune derived from an attempt to induce anergy into myelin-reactive T-cells in MS patients with the hope that this would be beneficial. The therapy, known as altered peptide ligand therapy, involves altering several amino acids within an antigenic peptide capable of activating T-cells. Alterations within the TCR contact regions can lead to T-cell inactivation when the altered peptide is presented to the T-cell. When Bielekova...
et al. (91) treated MS patients with a high dose of an altered peptide ligand of the major immunogenic epitope of human MBP\textsubscript{83–99}, instead of inducing anergy, 3 of 8 patients experienced expansion of their myelin-directed T-cells anywhere from 10-fold to 300-fold. All the three patients had a dramatic increase in MRI contrast-enhancing lesions and all three had clinical relapses. The results of this trial, which was halted early, directly link disease activity with an enhanced T-cell response to an autoantigen (MBP). This therapy is still undergoing investigation, but with modifications including lowered dose.

**HUMORAL IMMUNITY AS INDIRECT EVIDENCE FOR AUTOIMMUNITY IN MS**

The humoral arm of the immune system has been implicated in the pathogenesis of MS. The findings of oligoclonal bands (OCBs) and increased levels of intrathecal immunoglobulins (Igs) in more than 90% of MS patients strongly suggest involvement of B-cells in MS. The Igs found in MS CSF include IgG, IgA, IgM, and IgD (92). B-cells, plasma cells, and Ig are typically present in MS lesions, and at times have been identified in normal-appearing white matter of MS patients (93,94). Even in the very earliest cases examined, Ig and immune complexes have been observed consistently, suggesting a role for the humoral immune system from disease onset (95). An ongoing histological study of active MS lesions from biopsies and autopsies has found that the most common pattern of pathology involves Igs and complement, as well as mononuclear leukocyte infiltration (96).

Numerous studies have linked B-cells and antibody to MS prognosis. CSF cell phenotypes were assayed in 60 MS patients, and the results were correlated with clinical progression. Those patients displaying a “B-cell dominant” phenotype, with high percentages of B-cells, plasma cells, and IgG in CSF, had significantly faster disease progression ($r = 0.57; P < 0.0009$) than MS patients with a “monocytes dominant” phenotype (97). Increased concentrations of Abs in CSF of MS patients correlate with episodes of MS worsening (98). Excessive CSF free kappa light chains, a byproduct of Ig production, is correlated with poor prognosis (99). IgM and IgG in the CSF typically demonstrate a pattern of limited clonality, referred to as OCBs because of the banding pattern observed when concentrated CSF is electrophoresed through agarose. The presence or absence of CSF OCBs is correlated with MS prognosis. Patients lacking CSF OCBs typically have a more benign course (100). Studies from this laboratory suggested that higher numbers of CSF-specific OCBs at MS onset is associated with poorer clinical outcome (101).

Although CSF IgG is typically the only Ig isotype measured by clinical laboratories, published studies indicate that CSF IgM levels and OCBs composed of IgM may also portend a worse prognosis, perhaps with better accuracy than magnetic resonance imaging (MRI) (102,103). The presence of IgM OCBs in the initial diagnostic spinal tap has been associated with both increased disability accumulation ($P < 0.002$) and with conversion to secondary progressive MS ($P < 0.0009$) (104).

Molecular studies indicate that production of Abs in the CNS of MS patients is antigen-driven, making an indirect case for autoimmunity. The complementarity-determining regions (CDR) of Abs are the antigen-binding sites, and include the Ig heavy-chain variable (VH) region. Somatic hypermutations occur in the CDR when B-cells are exposed to their antigen; these mutations often lead to amino acid substitutions that enhance Ig affinity for target antigen leading to “affinity maturation.”
In antigen-driven responses, mutations accumulate in Ig gene regions that contact antigen at a higher rate than in regions that have no antigen contact. In antigen-driven responses, mutations resulting in amino acid substitutions accumulate more than “silent” mutations. A number of studies have observed alterations typical of antigen-driven responses in CSF B-cells or B-cells in brain lesions of MS patients, indicating that B-cells have encountered their specific antigen in the CNS. These studies bolster the autoimmune hypothesis of MS pathogenesis.

Several groups of investigators have performed these studies with very consistent results. For example, in one study, IgG VH sequences from two acute MS plaques from a single patient were examined and compared with IgG VH sequences in subacute sclerosing panencephalitis (SSPE) brain and normal human brain. As expected, IgG purified from both the SSPE and MS brains displayed OCBs, whereas the normal human brain displayed a more heterogeneous Ig pattern. When the VH regions were cloned and sequenced, VH4 usage predominated within MS lesions, although the majority of sequences at the two sites from the one MS patient were different. All CDR sequences from the acute MS plaques displayed mutations compared to the germline (105). The same group later reported on studies of two additional MS brains where, once again, genes encoding Ig within MS plaques were more restricted in gene segment usage than germline, displayed multiple mutations, and had a high percentage of replacement mutations in the CDRs. This same pattern was noted in SSPE brain tissues, where there is a known antigenic stimulus, measles virus (106).

MOG is a minor protein component of CNS myelin, comprising less than 0.05% of myelin protein. However, this glycoprotein elicits a strong B-cell response (107), perhaps because MOG localizes to the outer surface of myelin and oligodendroglia. Humans can develop both cellular and humoral immune response to MOG (108–110). Arguably, B-cell and antibody responses to both MOG (111) and MBP (112) are somewhat more prevalent in MS patients than in controls. These antibodies may be the result, rather than the cause, of CNS pathology.

If the anti-myelin antibodies are critical to MS pathogenesis, they should be present at onset. Investigators have reported that in patients with a single isolated clinical demyelinating syndrome suggestive of MS, the presence of myelin-reactive IgM Abs in serum may predict the development of clinically definite MS. Of 103 patients initially presenting with neurologic symptoms suggesting demyelinating brain lesions evident on MRI, and OCBs in the CSF, serum samples were tested for Abs to MOG and MBP. Not all patients displayed anti-myelin antibodies, but those that did were more likely to have a second attack within two years than the seronegative patients. Those initially exhibiting both anti-MOG and anti-MBP Abs were most likely to have an early relapse (113).

Axonal damage is a common component of MS plaques, believed to be irreversible in the CNS. Neurofilaments are axonal cytoskeletal proteins. CSF antibodies against the 68 kDa light subunit of neurofilaments have been reported in the progressive forms of MS (114). Their presence in the CSF of MS patients has been correlated with lesion burden and cerebral atrophy, as detected by MRI (115). Cerebral atrophy in MS patients is thought to reflect diffuse axonal loss.

The above data constitute circumstantial evidence for the humoral immune response in MS pathogenesis. However, humoral immunity may not be completely detrimental in MS. Antibodies might also mediate CNS repair, as suggested by one group of investigators who have identified antibodies directed against oligodendrocytes, that appear to promote remyelination (116).
RESPONSE TO IMMUNOSUPPRESSIVE THERAPIES SUGGESTS 
AN AUTOIMMUNE ETIOLOGY

Immunomodulating and immunosuppressive therapies constitute the cornerstones of therapy for MS and will be discussed in detail in later chapters of this book. These agents include glucocorticoids, immunosuppressive agents such as mitoxantrone, immunomodulators such as interferon-β, and glatiramer acetate. All of these agents are partially effective and are thought to act by modulating the immune response. Glucocorticoids are useful for hastening recovery of acute relapses.

Glucocorticoids have a multitude of inhibitory effects on the immune system. They decrease expression of pro-inflammatory cytokines such as TNFα, IL-2, and IFN-γ (117,118). In most studies, they have been shown to increase expression of anti-inflammatory cytokines such as IL-10 and TGFβ-1 (119,120). Glucocorticoids also decrease MHC I and MHC II expression (121), induce T-cell apoptosis (122), inhibit nitric oxide synthesis (123), decrease expression of the adhesion molecules E selectin and ICAM-1 (124), decrease CSF matrix metalloproteinase 9 levels (125), decrease CSF IgG (126), and inhibit macrophage phagocytosis (127). Likewise, IFN-β induces a shift toward Th2 T-cell responses (128), inhibits T-cell activation (129), inhibits metalloproteinase-9 production (130), decreases Th1 cytokine levels (131), modulates adhesion molecule activity (130,132), and has other anti-inflammatory effects that are still being elucidated (133). Glatiramer acetate alters the Th1:Th2 balance toward Th2 cytokine production (134). Mitoxantrone, FDA-approved for relapsing-remitting and secondary progressive MS, is an immunosuppressive agent that decreases the number and activity of T- and B-lymphocytes and suppresses humoral immunity (135). The beneficial effects of these medications, which all inhibit or modulate the immune system, support the notion that MS is an immune-mediated disorder, perhaps initiated and sustained by autoimmunity.

THE AUTOIMMUNE HYPOTHESIS IS SUPPORTED BY 
ANIMAL MODELS

In 1935, Rivers and Schwenkter reported that an inflammatory demyelinating CNS disorder could be induced in monkeys with repeated injections of CNS tissue. The pathology of this disease had similarities to MS and its potential as a model for MS was immediately recognized (136). This model is known as experimental allergic (later autoimmune) encephalomyelitis (EAE). Although EAE does not identically replicate every aspect of MS, its similarities lend plausibility to the theory of autoimmunity as the cause of MS. Many components of the immune response in EAE have been corroborated in human MS, as will be discussed below. In the 1980s, it was demonstrated that this disease, induced readily in certain strains and species with whole spinal cord homogenate, could be induced with specific components of myelin (137). Later, Pettinelli and McFarlin (138) showed that CD4+ T-cells reactive with MBP could transfer the disease, confirming the primary role of T-cells. Moreover, following a single transfer of MBP-reactive T-cells, recipient mice displayed a relapsing-remitting phenotype (139). Zamvil et al. (140) demonstrated that the disease could be fully transferred by a single T-cell clone bearing a single T-cell receptor directed against an epitope of MBP. These studies unequivocally demonstrated that myelin-specific T-cells initiated this model for MS.
Similarities and differences exist between EAE and MS. EAE does not occur spontaneously in normal animals, but must be induced by evoking a strong anti-myelin cellular immune response. A notable exception is that when mice were created that were transgenic for a T-cell receptor (TCR) directed against MBP, on rare occasions EAE did occur spontaneously, but only when the mice were maintained in “dirty” housing conditions (141) or lacked any other functioning T-cells (142). Mice expressing a transgenic TCR in most of their T-cells are not representative of humans with MS. The course of EAE can be remarkably similar to certain clinical subtypes of MS. Some strains of mice, the SJL and PL strains in particular, have relapses and remissions, often remitting to neurologically normal between attacks. In mice with relapsing EAE, the frequency of relapses declines with time, as it does in MS (personal observations). For the SJL strain, female mice are more susceptible than males to EAE induction, another similarity to MS (143). Pregnant mice are less susceptible to EAE than nonpregnant littermates, reminiscent of the well-documented decline in MS activity during pregnancy (144). Susceptibility and clinical course of EAE are genetically determined, and linked to the MHC II, similar to MS (145). Some mouse strains, such as the C57BL/6, display a chronic EAE course without full recovery, but they seldom can be demonstrated to progress over time in the manner of primary progressive MS or secondary progressive MS. Histologically, EAE is similar to MS with inflammation comprising T-cells and macrophages, as well as smaller numbers of B-cells and plasma cells. Lesions are centered on blood vessels, much like MS. Murine EAE involves the spinal cord and optic nerves to a greater extent than the cerebrum, more similar in localization to neuromyelitis optica than to typical MS. Often an early wave of polymorphonuclear cells (PMNs) is seen during the initial hours of an EAE relapse (146). This is dissimilar to MS, as it is distinctly rare to observe PMNs in MS lesions. In a marmoset model of EAE with chronic relapsing disease, vesiculated myelin was observed in lesions, similar to some acute MS lesions. In both the marmoset AE model and in human lesions, myelin specific antibodies bound to areas of active demyelination were observed (147).

Most therapies that are effective in MS are effective in EAE as well. In fact, two therapies used in RRMS, glatiramer acetate and nataluzimab (under FDA review), were developed based upon data from the EAE model (148,149). Though not initially developed using the EAE model, beta-interferons are effective at inhibiting EAE (150). Despite limitations of the EAE model, its study has revealed a great deal about the development of an immune response within the CNS, and has led to new therapeutic agents for MS. The many similarities between EAE and MS support the case that MS is autoimmune in etiology. However, dissimilarities exist also. EAE is not MS, and some therapies that have clearly benefited certain models of EAE have not done so in humans. Inhibition of TNF-alpha is a case in point (151).

SUMMARY

The cause of MS is not known. Considerable indirect evidence points toward an autoimmune etiology. The data in support of an autoimmune cause for MS derive from investigators working worldwide in varied disciplines: genetics, cellular immunology, humoral immunology, and animal models. In addition, the beneficial clinical
and imaging responses observed in some MS patients to immunosuppressive agents is consistent with an autoimmune etiology. However, the autoimmune nature of MS remains unproven.

REFERENCES


INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) that affects approximately one million people worldwide and is the most common cause of nontraumatic disability in young adults (1). The cardinal features of the MS lesion, namely focal demyelination with relative axonal sparing, inflammation, and gliosis, were described and illustrated over 160 years ago by Carswell (1838), Cruveilher (1841), and Charcot (1868, 1880).

Although there is considerable heterogeneity in the clinical characteristics of MS, the disease is classified principally on the features of the clinical course at onset into “relapsing–remitting” or “primary progressive” (no attacks) (2). Relapses (exacerbations) are considered to represent the clinical correlate of recurrent episodes of inflammation and demyelination in the CNS, often accompanied by axonal injury. Recurrent attacks are commonly superseded by a phase of progressive disability thought to reflect a combination of ongoing demyelination, gliosis, and axonal loss. Remission of symptoms is likely due to remyelination and resolution of inflammation. A combination of both inflammatory and noninflammatory factors contribute to short- and long-term disability. The clinical predictors of natural history, however, are far from perfect, and there are no surrogate markers that accurately predict clinical course or outcome. Pathological features that clearly distinguish relapsing-remitting from progressive courses or favorable from poor prognoses in individual patients are not well defined. Furthermore, the biologic basis for the variable treatment response, often observed among MS patients, is not well understood and may reflect genetic, clinical, and/or pathologic heterogeneity. The advent of more sophisticated histological and molecular techniques to study MS pathology has provided new insights into the development and evolution of both focal and global tissue injury in MS. This chapter focuses on what we can learn about MS via detailed pathological analysis. The clinical and pathogenic relevance of these pathological studies is discussed.
HOW DOES STAGE OF DEMYELINATING ACTIVITY RELATE TO CLINICAL PHASE OF THE DISEASE?

The Chronic Inactive MS Lesion

The MS lesion may evolve differently during “early” and “chronic” phases of the disease. Different stages and types of demyelinating activity can be identified within these phases. Most neuropathological studies of MS are based on tissue from individuals with long-standing disease. Pathologically, these late chronic cases are characterized by the presence of multiple sharply demarcated plaques of demyelination typically ranging from <1 mm to several centimeters in size. Plaques are present in both white and gray matter, with a predilection for the periventricular white matter, optic nerves, brainstem, cerebellum, and spinal cord (3). By gross inspection, the plaques appear as circumscribed, slightly depressed gray colored areas with

![Figure 1](See color insert.) Chronic multiple sclerosis. Grossly, plaques appear as well-circumscribed, slightly depressed gray areas of increased tissue texture. The chronic inactive plaque microscopically appears as a sharply circumscribed area of myelin pallor (A, LFB/PAS) with variable reduction in axonal density (B, neurofilament protein). The lesions are hypocellular and lack macrophages containing myelin debris (C, KiM1P for macrophages). Abbreviation: LFB/PAS, luxol fast blue/periodic acid schiff.
increased tissue texture (Figure 1). The lesions may be round or oval, but frequently show finger-like extensions that may follow the path of small or medium sized vessels (4). Microscopically, the chronic inactive MS plaque appears as a sharply circumscribed, relatively hypocellular, pale area with marked myelin loss, prominent fibrillary astrocytosis, and variably reduced axonal density (Figure 1A–C). There is no evidence of active myelin-breakdown, and mature oligodendrocytes are markedly diminished or absent from chronic inactive lesions. Variable but usually scant chronic inflammatory infiltrates consisting of T-lymphocytes and macrophages may still be present, particularly in the perivascular regions.

The Active MS Lesion

On gross inspection, the active MS plaque appears as a cheesy soft area of irregular pink or gray color. Microscopically, active inflammatory demyelination is characterized by an intimate admixture of lipid-laden macrophages and large reactive astrocytes, accompanied by variable perivascular inflammation. The involved areas demonstrate marked pallor of myelin staining with “relative” preservation of axons, although where damage is most severe, axons may be lost or fragmented and display irregular tortuous and clubbed profiles (Figure 2A). Many macrophages become engorged with phagocytosed myelin remnants and debris and assume the appearance of classic “gitter cells” with abundant vacuolated cytoplasm. Intimately intermingled are enlarged (reactive) astrocytes with prominent, somewhat polymorphic nuclei and conspicuous eosinophilic cytoplasm. The so-called “granular mitosis” (also referred to as a Creutzfeld–Peters cell) is an unusual finding in some reactive astrocytes (Figure 2B). While resembling large chromosomes arranged like mitotic figures, they in fact represent small fragments of the nucleus (micronuclei). Although these cells are nonspecific and may be seen uncommonly in a variety of reactive processes, their presence should prompt consideration of active demyelination and should argue against the possibility of a glioma.

![Figure 2](See color insert.) Active multiple sclerosis lesion. Active lesions are hypercellular demarcated regions of myelin loss characterized by an admixture of macrophages and reactive astrocytes (A, LFB/PAS). Creutzfeld–Peters cells are astrocytes containing fragmented nuclei that resemble astrocytic mitoses (arrows A and B, H&E). Abbreviation: LFB/PAS, luxol fast blue/periodic acid schiff.
Stages of Demyelinating Activity

Although criteria for the pathological staging of MS lesions are controversial, in order to draw conclusions regarding the earliest events in the development of the MS lesion, it is critical that a precise definition for demyelinating activity be used. Some investigators rely on markers of inflammation to stage lesions based on the extent of perivascular or parenchymal inflammatory cell infiltration (5), the increased expression of histocompatibility antigens (6), or adhesion molecules (7,8), and the activation state of lymphocytes and macrophages within lesions (9,10). However, this definition does not distinguish demyelinating activity from inflammatory activity, which may be present even in the absence of ongoing active demyelination.

Active plaques have also been defined by the presence of cholesterol esters and neutral lipids in macrophages that stain positively for lipophilic dyes, such as oil red O or sudan II (sudanophilic stage of myelin degradation). The sudanophilic stage of myelin degradation, however, may persist for several months after the actual destruction of myelin sheaths (3), and therefore does not necessarily reflect the earliest events in lesion formation.

Magnetic resonance imaging (MRI) studies rely on evidence for blood–brain barrier leakage, defined by the presence of gadolinium; as an indicator of an active lesion (11,12). This may not reliably differentiate active from inactive MS plaques because both can be associated with variable degrees of blood–brain barrier leakage. MRI sensitivity may not be sufficient to detect potentially small quantitative differences in blood–brain barrier dysfunction that distinguish active from inactive plaques.

Gay et al. (13) developed a multifactorial cluster analysis method to stage lesion activity based on inflammation and microglial activation, immunoglobulin and complement deposition, demyelination, and parameters of the clinical history. Although this approach may help to identify stages of MS lesions that precede demyelination, reliable clinical details are not always available in a given case, and there often is a poor correlation between the clinical symptoms and the distribution of plaques because most conspicuous lesions occur in relatively silent areas of the brain.

A stringent definition of demyelinating activity within a plaque can be obtained by studying the structural profile and chemical composition of myelin degradation products within macrophages (10). Whenever myelin sheaths are destroyed, their remnants are taken up by macrophages or microglia cells. Minor myelin proteins, such as myelin oligodendrocyte glycoprotein (MOG) or myelin associated glycoprotein (MAG), are rapidly degraded within macrophages, within one to two days after phagocytosis. In contrast, major myelin proteins (MMP), such as myelin basic protein (MBP) and proteolipid protein (PLP), may persist in macrophages for 6 to 10 days. In later stages, the macrophages contain sudanophilic and periodic acid Schiff (PAS)-positive “granular lipids” that may persist in the lesion up to several months.

Stages of demyelinating activity can be defined as early or late active, inactive, or early and late remyelinating. “Early active lesions” are characterized by MOG+, PLP+, and luxol fast blue+ (LFB+) degradation products within the macrophage, as well as the expression of the acute-stage inflammatory macrophage markers, MRPl4 and 27E10 (Figure 3). “Inactive lesions” are characterized by hypocellularity, complete myelin loss, fibrillary gliosis, and variable reduction in axons. Inflammation may still be present with macrophages containing either empty vacuoles or PAS+ degradation products; “Early remyelinating lesions” contain small clusters of axons surrounded by thin myelin sheaths, and no myelin degradation products within
macrophages; variable inflammation and the presence of reactive astrocytes. Early remyelination may coexist with ongoing active demyelination. “Late remyelinating” (shadow plaques) represent focal areas of gliosis and reduced myelin density. In any MS brain, a variety of lesions at different stages of demyelinating activity may be present. When these stringent criteria are used, the incidence of active lesions in MS brains is low, especially in classical cases sampled during the chronic phase of the disease.

Types of MS Lesions

On the basis of topographical distribution of macrophages, and the type of myelin degradation products present within the macrophage, four types of MS plaques can be distinguished (Figure 4). The “acute active plaque” is characterized by the presence of macrophages containing early and late myelin degradation products, distributed throughout the extent of the lesion. The radially expanding “active rim” shows the accumulation of numerous macrophages, containing both early and late myelin degradation products, clustered at the advancing plaque edge, and diminishing in number toward the inactive plaque center. The plaque with low grade activity in a radial expanding “smoldering rim” is defined by the presence of manglia and

**Figure 3** (See color insert.) Early active demyelination. The early active lesion is characterized by the presence of LFB+ blue granules (A, arrow) and early myelin degradation products (B, arrows, MOG) within macrophage cytoplasm. Macrophages also stain positively for acute stage inflammatory macrophage markers (C, arrows; MRP14). **Abbreviations:** LFB, luxol fast blue; MOG, myelin oligodendrocyte glycoprotein.
very few macrophages restricted to the plaque edge containing early and late myelin degradation products (14). The “inactive plaque” contains no early or late myelin degradation products within the macrophages. Active plaques and active rims are mainly found in patients with acute or early MS, or in secondary progressive MS (SPMS) patients with ongoing clinical attacks. These plaques are usually associated with profound inflammation. Smoldering rims are mainly found in late phases of the disease, in particular, in patients with primary progressive MS (PPMS) or SPMS not associated with ongoing relapses (14). These plaques are typically associated with relatively less inflammation.

Figure 4  (See color insert.) Types of multiple sclerosis plaques (KiM1P, macrophage marker). (A) Acute active multiple sclerosis plaques are characterized by extensive macrophage infiltration throughout the extent of the lesion with macrophages containing both early and late myelin degradation products. (B) The radially expanding active rims consist of macrophages containing early and late myelin degradation products clustered at the advancing edge of the plaque, and diminishing in number towards the inactive plaque center. (C) Smoldering plaques are characterized by a low grade of demyelinating activity at the plaque edge, with very few macrophages containing myelin degradation products. (D) The inactive plaque is hypocellular and contains no early or late myelin degradation products within the macrophages.
The existence of a pre-active lesion derives from correlation of MRI and tissue pathologic features, and is largely based on a single MS patient who died in the active stage of the disease (15). Although numerous areas of MRI abnormality were present, only some revealed inflammation, blood–brain barrier damage, edema, and/or myelin pallor. This was interpreted as evidence of plaque development that preceded the overt dissolution of myelin sheaths. Although a pre-active lesion stage likely exists, it is doubtful that all brain lesions characterized by T2-weighted MRI abnormalities in which histological analysis reveals myelin pallor and some inflammation and edema are pre-active plaques. Both remyelinated shadow plaques and areas of Wallerian degeneration may show the identical pathological features that have been associated with the pre-active lesion, namely myelin pallor, edema, and inflammation. It seems likely that the earliest stages of demyelination depend on the identification of active myelin destruction and/or oligodendrocyte damage with myelin debris in macrophages.

WHAT IS THE PATHOGENIC ROLE OF INFLAMMATION IN MS?

Focal areas of myelin destruction observed in MS occur on a background of an inflammatory process dominated by the infiltration of T-lymphocytes, recruitment of hematogenous macrophages, the local activation of microglia, and the presence of relatively few B-lymphocytes or plasma cells (Figure 5). This inflammatory reaction is associated with the upregulation of a variety of cytokines within the MS

Figure 5  (See color insert.) Inflammation in multiple sclerosis lesions. The inflammatory infiltrate within an active multiple sclerosis lesion contains variable numbers of perivascular and parenchymal CD3+ T-lymphocytes (A), cytotoxic CD8+ T-lymphocytes (B), macrophages (C; KiM1P), CD20+ B-lymphocytes (D), and plasma cells (H&E).
lesion, including interleukin-1,2,4,6,10,12, gamma-interferon (γ-IFN), tumor necrosis factor alpha (TNF-α), and transforming growth factor beta (TGF-β) (16,17). Activated endothelial cells in active lesions express adhesion molecules, fibronectin, urokinase plasmin activator receptor, major histocompatibility complex (MHC) molecules, chemokines and their receptors, and stress proteins (18). In some MS patients, immunodominant peptides of MBP become complexed with DR2 molecules at sites of demyelination (19), and T-cell clones with receptors specific for MBP have been found in MS lesions (20). These observations, coupled with the pathologic similarities between MS and experimental autoimmune encephalomyelitis (EAE), suggest that MS is an autoimmune disease, initiated by MHC-class II-restricted CD4+ Th1 lymphocytes that produce pro-inflammatory Th1 cytokines. This leads to the recruitment and activation of hematogenous macrophages, which destroy myelin sheaths, either via toxic effectors or in co-operation with specific autoantibodies. The expression of these immune-associated molecules, however, is not specific for MS and can be seen in other T-cell driven processes of the nervous system, such as viral infections. Furthermore, the evidence that MS is a Th1-mediated disease remains indirect and circumstantial. Therapeutic strategies that are beneficial in EAE have often yielded ineffective, or at times, unexpected aggravation of MS (21). A likely explanation for this discrepancy may be that the pathogenesis of MS is more complex when compared with that of a pure Th1-mediated CNS autoimmune disease. Cells other than classical Th1 T-cells may contribute to MS pathology.

There is accumulating evidence that MHC class I-restricted T-cells may play an important role. The inflammatory infiltrates in MS lesions are dominated by class I MHC restricted CD8+ T-lymphocytes (13), and clonal expansions of T-lymphocytes are more pronounced for CD8+ compared with CD4+ T-lymphocytes (22). Myelin-specific CD8+ T-cells may even evoke EAE under certain conditions (23,24). Because antigen recognition by CD8+ T-lymphocytes requires the presentation of respective peptides in the context of MHC class I molecules, the expression of these molecules is most pronounced in acute lesions, followed by chronic active lesions and inactive lesions. Overall, all MS patients show more MHC class I expression compared with controls (25). Double staining and confocal laser microscopy reveals that in active MS lesions, class I expression is present not only on inflammatory cells, microglia, and endothelial cells, but also on astrocytes, oligodendrocytes, and some neurons and axons (25). These data suggest that all cell types in active MS lesions may become targets for class I restricted T-cell cytotoxicity (26). In support of these observations, axonal destruction in MS lesions correlates better with CD8+ T-cells and macrophages than CD4+ T-cells (27).

There is also evidence that Th2 cells can participate in pathologic autoimmune processes. Th2 polarized T-cells, directed against MBP, have been shown to induce destructive brain inflammation in immunodeficient mice (28). Similarly, circulating Th2 cells could drive the formation of antibodies in both MOG and MBP (29), which are present in MS lesions and in the serum of MS patients (30,31).

Despite the universal presence of inflammation in MS lesions, the pathogenic role of the inflammatory response is not clear. Neuropathologic studies reveal that inflammatory cells are not always present in areas of active demyelination, and persistent inflammation is a frequent and typical feature of chronic inactive MS lesions. In addition, active demyelination has been observed in immunosuppressed patients with little or no evidence of perivascular inflammation in the lesions (32). Finally, the abundance of inflammation in inactive cases, together with
recent observations on the local production of neurotrophic factors, such as brain-derived neurotrophic factor by leukocytes, may indicate an important role for inflammation in the repair of MS lesions (33). Interestingly, neurotrophin receptors are expressed on glial cells and neurons in or near actively demyelinating MS lesions (34). Autoimmune T-cells can protect optic nerve neurons after crush injury (35). Macrophages stimulate remyelination in tissue culture (36), while depletion of macrophages is associated with diminished remyelination (37). Therefore, there is likely a delicate balance between pathogenic and reparative factors that determine the final outcome of the MS lesion. Conceivably, the complete blockage of all inflammatory responses in the MS lesion could be counterproductive.

WHAT IS THE FATE OF THE OLIGODENDROCYTE AND EXTENT OF REMYELENIATION IN MS LESIONS?

Oligodendrocyte Pathology and Early Remyelination

Oligodendrocytes are susceptible to damage via a number of immune or toxic mechanisms present within the MS lesion. These include cytokines such as TNF-α (38), reactive oxygen or nitrogen species, excitatory amino acids such as glutamate (39), complement components, proteolytic and lipolytic enzymes, T-cell mediated injury via T-cell products (perforin/lymphotoxin) (40), the interaction of Fas antigen with Fas-ligand (41), CD8+ class I MHC-mediated cytotoxicity (42), or persistent viral infection (43). The fate of the oligodendrocyte in active demyelinating lesions is controversial. Some studies suggest an abundance of oligodendrocytes within the active MS lesion (44), whereas others report a partial reduction (45). Several previous studies suggested that the density of oligodendrocytes in actively demyelinating lesions varied between patients (9,46). The more recent availability of new markers to label oligodendrocytes in paraffin embedded formalin-fixed tissue has led to a systematic analysis of the density of oligodendrocytes within over 300 lesions from 113 patients with MS during the early phase of the disease (47). The numbers were correlated with stages of myelin degradation products within macrophages thereby providing a snapshot of the temporal evolution of the lesion. Oligodendrocytes were labeled with PLP mRNA, an early marker of oligodendrocytes actively engaged in myelin synthesis and maintenance, but not present in surviving oligodendrocytes that have lost their myelin sheaths. Cells were also stained with antibodies directed against MOG, which is expressed on the surface of myelin sheaths and terminally differentiated oligodendrocytes late in myelination. MOG is detectable on oligodendrocytes that have survived demyelination following Wallerian degeneration (48).

Two principal groups of oligodendrocyte pathology were identified in these early MS lesions (47). Group I (70% of the cases) was characterized by a variable (minor to moderate) reduction of oligodendrocytes at the active demyelinating plaque edge, with re-appearance of cells within inactive or remyelinated regions. These lesions were associated with prominent remyelination. Although markers for the identification of immature oligodendrocytes were not used, the presence of cells expressing PLP mRNA, but not MOG, suggests that these oligodendrocytes may have been derived from the progenitor pool. Group II (30% of cases) was characterized by extensive destruction of oligodendrocytes at active sites of demyelination in the absence of increased oligodendrocyte numbers in inactive plaque areas. In these lesions, remyelination was sparse or absent. Although there was profound heterogeneity of oligodendrocyte damage between patients, lesions from a single
individual, exactly matched for stage of demyelinating activity, showed very similar oligodendrocyte densities. Furthermore, the extent of early remyelination correlated with oligodendrocyte numbers within the lesion. Remyelination in early MS lesions is also associated with an increased expression of cell death inhibitory proteins such as bcl-2 (49). These studies suggest that early in MS, remyelination may be extensive and may occur simultaneously with demyelination (Figure 6). During the early stage of remyelination (myelin sheath formation), inflammation with prominent macrophage infiltration may be prominent within the lesion. The extent of remyelination at these early stages appears to depend on the availability of oligodendrocytes or their progenitor cells in the lesion. Furthermore, the profound heterogeneity in extent and topography of oligodendrocyte destruction in active demyelinating lesions suggests that myelin, mature oligodendrocytes and possibly oligodendrocyte progenitors, are differentially affected in subsets of MS patients. Different mechanisms of myelin and/or oligodendrocyte injury may be operating in an individual MS patient, and may thereby influence the likelihood of effective remyelination in the MS lesion.

Late Remyelination

Remyelination in chronic lesions may be restricted to the plaque edge or may extend throughout the lesion (Figure 7). Such lesions, referred to as shadow plaques, consist of sharply demarcated areas of complete remyelination, and are characterized by reduced staining of myelin (myelin pallor) due to a decreased ratio between myelin
sheath thickness and axonal diameter. These late remyelinating lesions typically contain few macrophages, and are typically associated with profound fibrillary gliosis. These remyelinated lesions may subsequently become targets of new demyelinating attacks (50).

The presence of cells in very early stages of oligodendrocyte development identified in completely demyelinated plaques devoid of mature oligodendrocytes, as well as in chronic lesions devoid of remyelination (51), suggests that the failure of remyelination at these later disease phases is not due to a lack of oligodendrocyte progenitors, as is the case in early remyelinating MS lesions, but rather the lesion microenvironment may not be receptive to remyelination signals (52). Whether this is due to an imbalance of growth factors, an abnormal composition of axons, glial scarring, or impaired axon-oligodendrocyte interaction is uncertain. To what extent progenitor cells already present within chronic MS lesions can be stimulated to divide, repopulate the lesion, and initiate remyelination must still be determined.

**IS THERE EVIDENCE FOR PATHOLOGIC HETEROGENEITY IN MS?**

MS is a heterogeneous disease with respect to its clinical, genetic, radiographic, and pathological features. Variability in treatment response among patients is not well understood. The limited efficacy of T-cell directed therapies may result from a failure to abolish inflammation or intervene more specifically, since neither the trigger nor target antigen are known. Nonetheless, much of MS research has focused on identifying a single cause and therapy effective for all patients.

Heterogeneity with respect to the character and extent of the inflammatory response, the pattern of demyelination, the nature of oligodendrocyte pathology, the extent of remyelination, and the degree of axonal injury and/or loss present in MS lesions is well recognized. This pathologic variability has largely been interpreted as resulting from the variable intensity of the inciting pathologic process. Nonetheless, although there is pathologic heterogeneity in MS lesions, there is a surprising degree of homogeneity with respect to these pathologic features within a single individual, when matched for demyelinating activity (9,46,47,53).
In most experimental MS animal models of EAE (e.g., rats, pigs, and primates), T-cell mediated immune responses against brain antigens result in inflammation, but only limited demyelination. This resembles the pathology of acute disseminated encephalomyelitis (ADEM), in which perivascular inflammation is dominant with minimal, if any, perivenular demyelination (54). These observations suggest that additional pathogenic factors are necessary to produce the widespread demyelination in MS, including, but not limited to demyelinating antibodies, cytokines and other soluble mediators, cytotoxic T-cells, reactive oxygen and nitrogen species, excitotoxic mechanisms, and mechanisms of primary oligodendrocyte injury (55).

In vitro and in vivo data imply that MS may be an umbrella term for several different pathogenic entities that unify on the vulnerability of the myelin and oligodendrocyte to a variety of immune and toxic mediators. This hypothesis has several implications. First, multiple terminal effector pathways may act in parallel within a single patient to produce the MS lesion, thus there would be little chance to treat or prevent MS by attempts to interfere with a single mechanism. Second, different patients or subgroups may have distinct dominant effector pathways leading to tissue injury, which are constant over time, and thus specific therapy directed toward a specific underlying mechanism would be possible provided subgroups of MS could be defined. Third, the dominant effector pathways in different patients or subgroups may change over time, thus specific therapies would need to be administered during specific disease phases. Fourth, different dominant pathways of tissue injury may produce the MS plaque early in disease; however, patterns eventually converge to a common mechanism responsible for ongoing demyelination and axonal injury in chronic phases. This would require the development of therapies that are specific not only for different pathological subtypes, but for different targets (myelin, oligodendrocyte, axon, and neuron), and different disease phases (early vs. chronic).

Detailed neuropathological studies on large numbers of active MS lesions (n = 82) have revealed a profound heterogeneity in immunopathologic patterns of demyelination (53,56). Although all active lesions occur on a background of an inflammatory process, composed mainly of T-lymphocytes and macrophages, the lesions segregate into four dominant patterns of demyelination based on plaque geography, extent and pattern of oligodendrocyte pathology, evidence for immunoglobulin deposition and complement activation, and pattern of myelin protein loss (Figure 8).

The four patterns are:

Pattern I: Macrophage associated demyelination

Pattern II: Macrophage associated demyelination with local precipitation of immunoglobulins and activated complement (antibody associated demyelination)

Pattern III: Demyelination with primary alterations in the most distal oligodendrocyte processes and oligodendrocyte apoptosis (distal dying-back oligodendrogliopathy associated demyelination)

Pattern IV: Primary degeneration of oligodendrocytes in the periplaque white matter with secondary myelin destruction (primary oligodendrogliopathy).

In both patterns I and II, macrophages and T-cells predominate in well-demarcated plaques that surround small veins and venules; however, pattern II lesions demonstrate the local precipitation of immunoglobulin and activated complement in regions of active myelin breakdown. The expression of all the myelin
Figure 8  (See color insert.) Immunopathological patterns of early multiple sclerosis lesions. Pattern II active lesions are well demarcated from the peri-plaque white matter (A; LFB/PAS), contain numerous macrophages (D; CD68), and are characterized by an equal loss of MOG (B) and MAG (C) immunoreactivity. Pronounced deposition of C9neo-antigen is present on degenerating myelin sheaths and within myelin degradation products taken up by macrophages in the zone of active demyelination (E; C9neo). Pattern III lesions are ill-defined (F; LFB/PAS), contain numerous macrophages (I; CD68), and demonstrate a preferential loss of MAG (H) immunoreactivity relative to MOG (G). Apoptotic oligodendrocytes are present within active pattern III multiple sclerosis lesions (J, K: arrows; CNP-ase). Abbreviations: LFB/PAS, luxol fast blue/periodic acid schiff; MAG, myelin associated glycoprotein; MOG, myelin oligodendrocyte glycoprotein. Source: From Ref. 53.
proteins (MBP, PLP, MAG, and MOG) are reduced similarly. Oligodendrocytes are reduced in number at the active edge, but re-appear within the plaque center. Remyelination is often extensive. Pattern I (macrophage associated demyelination) closely resembles myelin destruction in mouse models of autoimmune encephalomyelitis in which mainly toxic products of activated macrophages such as TNF-α (57) and nitric oxide (58) mediate destruction of myelin sheaths. Lesions similar to pattern II (antibody associated demyelination) are found in models of EAE, induced by sensitization with MOG. In this model, demyelination is induced by a cooperation between encephalitogenic T-cells and demyelinating anti-MOG antibodies (59). Although pattern II lesions suggest antibody (Ab) and complement mediated mechanisms may contribute to demyelination and tissue injury, definite proof is still lacking. A study describing deposition of MOG-reactive Igs on degenerating myelin sheaths in an active MS lesion (30) provides some support for this notion.

Pattern III lesions are defined by oligodendrocyte apoptosis, a marked reduction in oligodendrocytes, minimal remyelination, and early loss of MAG and 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) myelin proteins. The pronounced reduction in the expression of MAG and CNPase, myelin proteins localized to the most distal extension of the oligodendrocyte cell body—the periaxonal region—has been described in some MS lesions since the early 1980s (60,61). A similar selective loss of MAG has been described at the periphery of progressive multifocal leukoencephalopathy lesions, a known viral infection of glial cells, particularly oligodendrocytes, in which the infected cells are unable to maintain their myelin sheaths (62). Thus, a pattern of demyelination in which the destruction of MAG precedes that of the major myelin proteins (MBP, PLP) suggests a process at the level of the oligodendroglial cell body, and is consistent with a distal dying-back oligodendrogliopathy, in which the cell body is unable to support the metabolic demands necessary to maintain the distal axon. Ultrastructurally, this pattern is characterized by alterations in the distal-most extensions of the oligodendroglial processes, the periaxonal region, with a uniform widening of inner myelin lamellae and degeneration of inner glial loops antedating destruction of the myelin sheaths. These pathological alterations have been described in certain experimental models of toxin and viral induced demyelination, as well as in several stereotactic brain biopsies obtained for diagnosis in cases of early MS (63–65).

The preferential loss of MAG, a hallmark of pattern III lesions, is also observed in acute white matter ischemia (66). Prominent nuclear expression of hypoxia inducible factor (HIF)-1α, a specific and sensitive marker for hypoxia-like metabolic injury, also occurs in pattern III. This shared expression of HIF-1α in acute ischemic lesions and pattern III MS lesions suggests that a hypoxia-like metabolic injury may contribute to the pathogenesis of inflammatory white matter damage in a subset of MS patients (67). Microarray analysis of normal appearing white matter (NAWM) from 10 post-mortem MS brains revealed upregulation of genes, such as HIF-1α, known to be involved in neuroprotective mechanisms induced by hypoxic preconditioning (68). Whether this upregulation reflects an adaptation of cells to the chronic progressive pathophysiology of MS, or the activation of neuroprotective mechanisms in response to ischemic preconditioning in a subset of patients remains to be determined. Pattern III lesions are ill-defined, typically do not surround blood vessels, and do not demonstrate evidence for immunoglobulin or activated complement. The lesions are invariably associated with T-cell dominated inflammation and microglial activation. However, the quality of microglia activation is different from that observed in other MS lesions. Pattern III microglia highly express inducible nitric oxide synthase

Lucchinetti and Parisi
(i-NOS), but lack other activation markers (e.g., CCR5 and CD14) (69). Since both the pattern of demyelination and microglial activation resembles that found in acute white matter infarction, pattern III lesions may develop on a background of histotoxic hypoxia, perhaps due to mitochondrial damage induced by oxygen or nitric oxide radicals.

Pattern IV lesions, the least common, are associated with profound nonapoptotic oligodendrocyte death in the periplaque white matter. Since these lesions are very rare and identified only in autopsy cases, their pathogenesis is unclear.

The frequency of immunopathological patterns in 286 demyelinating disease cases (238 biopsies; 48 autopsies) analyzed to date, reveal a distribution similar to previously published studies. (15% pattern I, 58% pattern II, 26% pattern III, and 1% pattern IV) (Figure 9) (70).

The concept of pathological heterogeneity in MS lesions is further supported by immunocytochemical studies quantifying chemokines, including cellular

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**Figure 9** (See color insert.) Schematic representation of the four different multiple sclerosis immunopathological subtypes based on the underlying mechanism of myelin/oligodendrocyte destruction. Patterns I and II have sharp macrophage (MΦ) borders, surround vessels, and show oligodendrocyte preservation and remyelination. Pattern II also has complement and Ig deposition. Pattern III shows ill-defined MΦ borders, myelin associated glycoprotein loss (arrow), oligodendrocyte apoptosis, distal dying back oligodendrogliopathy with inner glial loop degeneration and limited remyelination, while IV shows oligodendrocyte degeneration in the white matter. **Source:** From Ref. 163.
expression of CCR1 and CCR5 in pattern II \( (n = 21) \) and pattern III \( (n = 17) \) lesions relative to demyelinating activity \((69)\). Infiltrating monocytes in lesions of all patterns co-express CCR1 and CCR5. In pattern II, the number of CCR1 cells decreases, while the number of CCR5 expressing cells increases in late active versus early active regions. In contrast, CCR1 and CCR5 cells are equal in all regions of pattern III lesions and resembles the expression pattern seen in acute strokes. These data support the notion of distinct inflammatory microenvironments in pattern II and III lesions, and suggest pathological heterogeneity in MS lesions.

**DOES PATHOLOGIC HETEROGENEITY REFLECT PATHOGENIC HETEROGENEITY IN MS?**

The demonstration of pathologic heterogeneity in early MS lesions must not be taken as confirmatory evidence for pathogenic heterogeneity among MS patients. This distinction is important as there is a lack of consensus regarding whether lesion heterogeneity can exist within the same patient and is stage-dependent \((71)\), or, in contrast, is patient-dependent and reflects distinct pathogenic subtypes of MS with a dominant effector mechanism of tissue injury operating within a given patient early in the disease \((70)\).

**Pathologic Heterogeneity Is Stage Dependent vs. Patient Dependent**

Barnett and Prineas, on the basis of finding extensive oligodendrocyte apoptosis in the absence of inflammation in a MS patient who died nine months after disease onset, and 17 hours after presentation with acute pulmonary edema, suggested that primary oligodendrogliopathy represents the initial lesion in relapsing–remitting disease, preceeding inflammation and active myelin breakdown \((71)\). A combination of lesions, some showing remyelination and others complement activation, was interpreted as evidence of an overlap of pathological features that have been associated with patterns II and III, respectively. The authors proposed that immunopathological heterogeneity resulted from an initial lesion formed from a primary oligodendrocyte insult (resembling pattern III) followed by remyelination, and then an additional attack of active demyelination associated with complement activation (similar to pattern II) \((71,72)\). This sequence of events could explain the coexistence of complement activation, remyelination, and oligodendrocyte apoptosis in this unique case, and challenges the hypothesis of pathogenic heterogeneity within MS patients.

It is important to note that since lymphocyte subsets were not examined, a role for inflammation in these lesions cannot be completely excluded. Other confounding features of this case include treatment with high dose corticosteroids prior to death, which may have dampened the inflammatory response, and the presence of probable hypoxia related to the patient’s known perimortem pulmonary edema, which is known to result in an identical pattern of myelin and oligodendrocyte pathology \((66)\).

An alternative view of patient-dependent immunopathological patterns has been advocated by Lucchinetti et al. \((53)\). On the basis of detailed analyses of over 286 immunoclassified cases, no remnants of “pre-existing” pattern II lesions exhibiting fresh activity or overlapping of immunopathological patterns among actively demyelinating areas from a single lesion of a given patient have been noted \((70)\). Although lesion patterns differed between patients, the pattern classification was identical among all active lesions examined from a given patient \((70)\). In addition,
all pattern III cases \((n = 76)\) were associated with inflammation, in the setting of active myelin breakdown with no evidence for complement activation.

**Clinical–Paraclinical Correlates**

If immunopathological patterns evolve over time, an association between the immunopattern and time from onset to biopsy/autopsy would be expected. Preliminary data on a large sample size \((n = 75, \text{ biopsies with face-to-face clinical assessment})\), however, have shown no such correlation. Although it is difficult to determine lesion duration prior to symptom onset or biopsy, and if a lesion is clinically symptomatic, most cases underwent biopsy within days to weeks of symptom onset (median 1 month, range 0.1–15 months). Assuming that all active lesions begin with a single pattern, one would expect to find an over-representation of pattern III lesions in cases with the shortest interval between onset and biopsy, which was not found in our material to date. In patients biopsied up to four years after onset, and in autopsy cases dying after more than five years disease duration, a similar distribution of immunopathological patterns was noted. These findings challenge the assertions of Barnett and Prineas and suggest that heterogeneous patterns of myelin destruction can be identified at later MS phases. However, it is necessary to confirm these data, and additional immunopathologically classified biopsy and autopsy cases with sufficient clinical data to ascertain disease duration prior to tissue sampling need to be examined.

The hypothesis of pathogenic heterogeneity in MS may be important for future studies on the etiology and therapy of the disease. The potential to apply these findings to MS patients requires the development of strategies that allow the stratification of MS pathologic subtypes without being dependent on brain biopsies. Immunopathologic specific clinical and paraclinical surrogate markers need to be identified. A detailed clinical follow-up of the biopsy cohort \((n = 99 \text{ patients})\) has failed to reveal any correlation between time of symptom onset, date of biopsy, and pathological pattern (73). In addition, a striking correlation between therapeutic response to plasma exchange in MS patients with evidence for antibody and complement activation (pattern II pathology, \(n = 10\)) on biopsy, compared to no response in pattern I \((n = 3)\) or pattern III \((n = 6)\) cases, has been observed, suggesting this classification scheme may have important pathogenic and treatment implications (74). Neuroimaging studies suggest the sharp border at the active plaque edge with accumulating macrophages typical of active lesions in pattern I and II. MS lesions is highly associated with the presence of ring enhancement on gadolium (Gd)-MRI and hypointense T2 rims, whereas these imaging features are not found in pattern III lesions \((P < 0.001; \text{ 54 cases examined})\) (75). In addition, review of follow-up MR images from pattern III cases \((n = 13)\) has not revealed ring enhancing lesions thus far, further supporting the hypothesis of pathogenic heterogeneity. If lesion heterogeneity reflected a stage-dependent phenomenon, one would expect a ring enhancing lesion in pattern III cases. Additional studies with longer follow-up are needed before concluding that there is pathogenic heterogeneity in immune effector mechanisms involved in MS lesion formation, rather than a single mechanism dominating the formation of all lesions. Since the immunopattern classification scheme developed by Lucchinetti et al. relies on identifying active MS lesions defined by the presence of macrophages containing early myelin degradation products, and as such is largely derived from early biopsy or acute autopsies, it is unknown whether immunopathologic heterogeneity persists in the slowly expanding smoldering rims typically seen in established MS patients with longstanding active disease.
WHAT IS THE SUBSTRATE OF IRREVERSIBLE DISABILITY IN MS?

The accumulation of irreversible impairment and disability in MS is believed to occur via two mechanisms. Attack-related disability may occur in patients with relapsing MS (i.e., RRMS or SPMS with ongoing exacerbations). Although most MS patients recover after an exacerbation, some may experience a step-wise decline in neurologic function. In one study, approximately 40% of patients had residual deficit of at least 0.5, and 28% had residual deficit of ≥1 expanded disability status scale (EDSS) units on an average of 64 days after an exacerbation, suggesting that MS exacerbations produce a measurable and sustained effect on disability (76). On the other hand, progression-related disability may occur in patients with progressive forms of the disease (i.e., PPMS or SPMS with or without superimposed exacerbations) and is characterized by gradual decline in neurologic function that occurs independent of clinical exacerbations or MRI evidence of lesion activity.

Both relapse-related and progression-related disabilities may eventually lead to permanent neurological impairment; however, the pathologic mechanisms leading to this irreversible disability may be different. Furthermore, a complex relationship exists between inflammatory demyelination and neurodegeneration in MS. To what extent these pathologic processes may occur independent of one another must be clarified in order to develop effective therapeutic strategies that limit the accumulation of disability in MS. Pathological, clinical, experimental, and neuroimaging studies provide important clues, which may help dissect this complex relationship.

THE INFLAMMATORY DEMYELINATION/NEURODEGENERATION PARADOX

Clinical Studies

The apparent dissociation between inflammation, demyelination, and axonal injury are supported by clinical studies. Four trials of interferon beta (IFN-β) have been published in SPMS (77–80). Although all of these demonstrated a benefit on relapses and MRI activity, the results on disability progression differed among the trials. In the European Interferon Beta (IFN-β)-1b study (IFN-β), treatment slowed worsening on the Kurtzke expanded EDSS, whereas in the other three, no benefit was seen on this endpoint. A metanalysis of the four trials revealed that patients with recent relapses and rapid decline were more likely to benefit from (IFN-β) treatment, with slowing of disability progression, than patients with remote relapses and slow gradual progression. Relapse frequency in the early phase of the disease influences time to onset of progression; however, once a threshold of disability is reached, rate of progression of disability is not affected by relapses either before the onset of the progressive phase or during this phase (81,82). During the progressive phase, the rate of clinical deterioration is similar between SPMS and PPMS patients (81,82). The absence of a relation between relapses and irreversible disability suggests a dissociation at the biologic level between recurrent acute focal inflammatory demyelination and progressive CNS degeneration. This apparent paradox is consistent with the persistence of disability progression in MS patients, despite infection with HIV (83) or suppression of cerebral inflammation after treatment with a potent antileukocyte monoclonal antibody (84). It is also in keeping with the refractory state of progressive MS to
anti-inflammatory therapies (85,86). These findings imply that therapeutic agents that have a short-term effect on MS relapses may not necessarily delay the development of long-term disability. Therefore, a combination of both inflammatory and noninflammatory factors likely contribute to disability in MS.

MRI Studies

MRI reveals that axonal loss in MS lesions correlates with the presence of permanent T1-weighted “black holes” on MRI (87–90), a reduction in N-acetyl aspartate (NAA) on magnetic resonance spectroscopy (MRS) (91,92) and the extent of CNS atrophy in the spinal cord (93). Since these imaging parameters correlate with clinical disability, axonal damage likely contributes to irreversible clinical disability in MS.

New Gd enhancing lesions mainly occur during the early relapsing phase of the disease, whereas Gd-enhancing lesions occur less frequently during the slowly progressive phase of the disease, characteristic of PPMS and SPMS without relapses (94). There are also progressive signal abnormalities in the so-called “normal appearing” white matter of MS patients. MRS reveals reduced NAA and elevated creatine levels in the NAWM of primary progressive MS (95,96), and magnetic transfer ratios are reduced in the NAWM of chronic versus relapsing disease (97). These changes have been interpreted as evidence of axonal destruction in the white matter plaques leading to secondary (Wallerian) degeneration (92,98,99). However, brain atrophy in MS is, in part, independent of T2 lesion load, suggesting that NAWM pathology not only reflects Wallerian degeneration of axons traversing macroscopic lesions, but also reflects microscopic or diffuse lesions not detected by MRI (100). Diffuse white matter damage and axonal loss can be severe despite very few white matter lesions (101,102), again highlighting the dissociation between inflammatory demyelination and neurodegeneration in MS. The focus on focal white matter pathology in MS represented by enhancing and nonenhancing lesions on MRI may have missed potential pathological differences between relapsing and progressive MS. Defining these pathologic differences may help better understand the dissociation between inflammatory demyelination (relapses/MRI activity) and neurodegeneration (gradual disease progression) in MS.

Experimental Studies

Experimental systems reveal that inflammatory demyelination, axonal loss, and neurologic disability can be dissociated. Mice deficient for the MHC class-I light chain, β2 microglobulin, develop inflammatory demyelination, but no early axonal damage or clinical deficit, following Theiler’s virus infection (103,104). These data suggest that the mechanisms underlying inflammatory demyelination and axonal injury may in part have separate pathogenic bases.

Pathological Studies

Axon Pathology

Although the MS lesion includes both inflammatory and demyelinating components, their relative influence on axonal loss is unclear. Classical neuropathologic descriptions of Charcot (1880), Marburg (1906), and Doinikow (1915) recognized degeneration of axons in MS lesions, but emphasized the primary demyelinating nature of the disease (105). More recent studies have demonstrated a high incidence of acute
axonal injury within both chronic and early MS lesions (106–108), although the extent of axonal injury is variable, ranging from 20% to 90% reduction in axonal density relative to the periplaque white matter (Figure 10). Furthermore, there is interindividual heterogeneity in the extent of acute axonal injury (27). Pathological studies reveal that myelin and axonal pathology may occur independent of one another. Ongoing axonal injury is present in inactive plaques, and the damage to axons does not seem to depend on the stage of demyelinating activity (27). Furthermore, acute axonal injury can be found in the normal appearing and periplaque white matter of MS patients.

Data are limited on the mechanisms of axonal injury (Figure 11). The extent of axonal transection in early active lesions correlates with inflammation, therefore

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure10}
\caption{(See color insert.) Axon loss in multiple sclerosis. Axonal density is reduced at the plaque edge and the plaque center, relative to the PPWM (A; neurofilament protein). Some of the reduced neurofilament staining at the active plaque edge can be attributed to macrophage infiltration. Consistent with acute axonal injury are numerous enlarged axonal profiles, axonal spheroids, and fragmented axons within the lesion (B, amyloid precursor protein; C, Bielschowsky silver). Abbreviation: PPWM, periplaque white matter.}
\end{figure}
during the acute stages of the disease, inflammatory mediators likely contribute to axonal injury. An association between the numbers of CD8+ T-cells and the extent of axon damage has been reported (27), and experimental studies implicate a CD8-MHC class I mediated pathway of axon destruction (103). Furthermore, the attachment of activated CD8+ T-cells containing cytotoxic granules polarized toward the demyelinated axon suggest direct CD8+ T-cell mediated cytotoxicity (26). Macrophages and microglia are often found in close contact with degenerating axons. Toxic inflammatory mediators liberated from these cells, such as proteases, cytokines, and free radicals, including nitric oxide (NO), may also lead to axonal injury. At low concentrations, NO induces a functional conduction block, but at higher concentrations, NO derivatives may irreversibly damage axons, particularly when they are electrically active (109). Axon-specific antibodies and complement may also be involved in mediating axonal injury. Anti-ganglioside antibodies were found to be significantly higher in PPMS than in SPMS or RRMS (110). Axons exposed to complement after demyelination may directly activate the complement cascade (111).

The magnitude of axonal loss in chronic lesions suggests mechanisms other than inflammatory demyelination may contribute to axonal damage during these later disease phases. Extensive acute axonal injury occurs during early stages of demyelination; however, a slow ongoing axonal destruction is also present in inactive MS lesions that lack inflammation (106). Although only a few axons are destroyed at a given time point, such lesions may persist in the CNS for years.
repeated demyelination within previously remyelinated lesions may contribute to axonal loss in chronic MS (50). Chronically demyelinated axons may also degenerate due to the lack of trophic support from myelin and oligodendrocytes. Mice lacking certain myelin proteins (MAG and PLP) demonstrate late onset axonal pathology, as well as evidence for an increased incidence of Wallerian degeneration (112,113). Secondary (Wallerian) degeneration also contributes to diffuse axonal loss (114).

The mechanisms of axonal destruction in MS may vary depending on the phase of the disease. In early phases axonal injury correlates with inflammation, whereas during later phases this correlation is less evident. This might explain the benefit of anti-inflammatory and immunomodulatory agents on early relapsing MS, with limited, if any, benefit on gradual disease progression.

Once axonal injury has been triggered, a cascade of downstream mechanisms ultimately leading to axonal disintegration occurs (114). These mechanisms are similar in a variety of pathologic conditions including inflammation, ischemia, and trauma. Acute axonal injury leads to a disturbance in the axoplasmic membrane permeability and subsequent energy failure leading to uncontrolled sodium influx into the axoplasm, which reverses the sodium/calcium exchanger and results in excess intraxon calcium. This activates Ca\(^{2+}\)-dependent proteases, which degrade cytoskeletal proteins, further impairing axonal transport. Voltage gated calcium channels (VGCC) accumulate at sites of disturbed axonal transport, leading to further Ca\(^{2+}\) influx, and eventually dissolution of the axonal cytoskeleton and axonal disintegration. Therapeutic strategies that inhibit different steps in the execution phase of axonal destruction, such as Na\(^{+}\) channel blockers, inhibitors of the Na\(^{+}\)–Ca\(^{2+}\) exchanger, blockade of VGCCs, or inhibition of calcium-dependent proteases, may help limit axonal destruction in MS. Clinical trials are needed with these agents to determine whether they slow disease progression.

Gray Matter Pathology

By concentrating on focal white matter lesions, previous neuropathological studies have not found major differences between patients with relapsing or progressive disease (115). However, there are pathological alterations in both the gray matter and NAWM of MS patients who contribute to disability.

MS may involve the gray matter, either as a classically demyelinated plaque or as neuronal loss and atrophy following retrograde degeneration from white matter lesions (3). Demyelinated plaques may be found in deep cerebral nuclei (116), or in the cerebral cortex (3). Cortical plaques are a well recognized but variable feature of MS pathology. Three types of cortical lesions can be distinguished: intracortical perivascular lesions; cortico-subcortical compound lesions affecting gray and white matter; and surface oriented band-like cortical lesions (117–120). The first two types develop around small veins and venules, whereas the third type is characterized by demyelination of the outer three to five layers of the cortex, resulting in band-like demyelinated lesions spanning several millimeters or centimeters of the cortical surface (118). This latter cortical lesion is the most common. These cortical lesions have a predilection for the cortical sulci, as well as the cingulate, temporal, insular, and cerebellar cortex. The lesions are associated with inflammatory infiltrates in the meninges.

Although cortical plaques share some pathologic features with white matter plaques, including demyelination, relative axonal and neuronal preservation, and some remyelination, they differ in several fundamental respects (118). The lesions tend to be less inflammatory, and blood–brain barrier damage is negligible, even
when the lesions are in the stage of active demyelination. A quantitative study of T- and B-cell infiltrates showed no significant differences between the normal cortex of control patients and those with MS or demyelinated lesions in the cortex (119). However, cortical plaques are associated with massive activation of cortical microglia (119), and very high expression levels of i-NOS. Cortical lesions tend to be associated with less tissue destruction, likely due to the limited amount of myelin, coupled with the limited axonal and neuronal injury.

The degree of cortical involvement and whether it correlates with clinical course or disability in MS are unknown. Cortical demyelination could impact neuronal, dendritic, and axonal function, viability, and survival. A recent study demonstrated the presence of apoptotic neurons within the demyelinated cortex (118). This may be relevant to the pathogenesis of neurologic and cognitive disability in MS and could, in part, explain why the disease progresses in PPMS in the absence of extensive white matter abnormalities. Degeneration of cortical neurons could also partly explain the diffuse NAA loss observed in the NAWM of PPMS patients. Furthermore, cortical damage could lead to secondary tract degeneration, which may account for some of the diffuse spinal cord changes observed in PPMS. Besides demyelination, the cerebral cortex of MS patients may also be affected by tissue loss and atrophy, particularly at sites of severe focal or diffuse white matter injury. Neurons in such lesions may show signs of retrograde reaction, such as central chromatolysis. Quantitative MRI analyses show that cortical atrophy may occur early and to some extent predicts the clinical course and the development of cognitive impairment (121). Furthermore, degeneration of cortical neurons could contribute to the diffuse NAA loss described within both the NAWM and spinal cord. Recent observations suggest that patients with SPMS and PPMS contain a larger number of cortical lesions as compared to RRMS (122). These observations may explain why the disease progresses in PPMS in the absence of extensive white matter abnormalities.

NAWM Pathology

Previous studies have been limited in their ability to correlate functional neurological deficit with focal white matter lesions determined by quantitative MRI techniques. This is particularly the case in PPMS patients, in which severe neurological deficits are associated with a surprisingly low lesion load in the brain (101,123) and spinal cord (124,125). Although diffuse NAWM injury is in part due to axonal transaction within plaques leading to secondary (Wallerian) degeneration, recent MRI data indicate that extent of tissue damage within focal plaques does not fully explain the degree of diffuse white matter changes (126,127), but suggest that global permanent neurological deficit may be determined by global and diffuse changes in NAWM (98,128–130).

There are few pathological studies of the NAWM in MS. Many have described mild inflammation (mainly CD8+ T-cells), microglial activation, gliosis, increased expression of proteolytic enzymes within astrocytes and microglia, diffuse axonal injury, and nerve fiber degeneration (106,131–135). A recent study compared the global brain damage in acute, relapsing, and progressive MS, and found a diffuse inflammatory process characterized by perivascular and paranechymal inflammatory infiltrates in progressive, but not acute or relapsing disease (122). The extent of inflammation was distributed globally throughout the brain, and was associated with widespread microglial activation characterized by CD68 expression, a marker for phagocytic activity, as well as MHC class II antigen and iNOS expression. Despite
the lack of primary demyelination in the “normal” white matter, axonal spheroids and terminal axonal swellings were variably present throughout the tissue. The extent of inflammation and axonal injury in the NAWM, as well as the degree and character of cortical demyelination, did not correlate with the number, distribution, activity, or destructiveness of focal white matter lesions (122).

The Relationship Between Focal White Matter Lesions, Global Tissue Injury, and Clinical Course in MS

These observations suggest that there are three basic pathologic processes in MS. The hallmark of acute or relapsing MS is the focal inflammatory demyelinated white matter lesion, whereas the hallmark of chronic progressive MS additionally includes diffuse “NAWM” damage and cortical demyelination. These three pathological processes occur in parallel, as well as independent from one another, as supported by the lack of correlation between plaque load in the white matter and the extent and character of cortical demyelination or NAWM injury. These pathological observations appear consistent with MRI studies, which suggest a dissociation between white matter lesion load and diffuse global pathology in MS patients.

The substrate of disability in MS likely varies in relation to the phase of the disease. Regardless of the course or phase of the disease however, neurodegeneration in MS appears to occur on a background of inflammation. Early axonal loss within the MS lesion contributes to relapse-related disability. This injury correlates with the degree of inflammation in the lesion. Late axonal loss subsequently occurs distal to the lesion, as a consequence of Wallerian degeneration. This secondary tract degeneration may contribute to the gradual slow progression seen in most MS patients. The presence of global brain injury, involving the cortex and “NAWM”, occurring diffusely and independent of focal white matter pathology may also contribute to the gradual progression of disability in MS.

Focal new white matter lesions are associated with blood–brain barrier damage, inflammation, and acute axonal injury both in the lesion, as well as distal to the lesion site due to Wallerian degeneration. This type of injury is likely to be limited by immunomodulatory and immunosuppressant drugs. However, diffuse global brain injury is associated with a compartmentalized inflammatory response that occurs typically behind an intact blood–brain barrier in the absence of ongoing focal white matter demyelination. Brain inflammation in slowly progressive MS is typically not associated with blood–brain barrier damage. There is no expression of blood–brain barrier disturbance markers on endothelial cells, and MRI studies typically demonstrate an absence of Gd-enhancing lesions in PPMS or non-relapsing SPMS (136). The limited benefit of anti-inflammatory or immunomodulatory therapy in the chronic, slowly progressive phase of MS may in part be explained by the compartmentalization of this inflammatory reaction in the CNS.

WHAT IS THE SPECTRUM OF IDIOPATHIC INFLAMMATORY DEMYELINATING DISEASES?

A variety of inflammatory demyelinating CNS disorders result in the structural hallmarks of the MS lesion, namely, demyelination, inflammation, and variable axonal loss. These disorders represent a broad spectrum of disease with variable clinical course, regional distribution, and pathology, and include the fulminant demyelinating
disorders [Marburg variant of acute MS, Balo concentric sclerosis (BCS), and ADEM]; the monosymptomatic idiopathic inflammatory demyelinating disorders (transverse myelitis, isolated optic neuritis or brainstem demyelination); and the recurrent disorders with a restricted topographical distribution [Devic neuromyelitis optica (NMO), and relapsing myelitis] (137).

**Marburg MS**

The Marburg variant of MS, recognized as a fulminant and lethal subtype of multiple sclerosis by Otto Marburg in 1906 (138), is characterized by rapid progression and an exceptional severity. The course is generally monophasic and relentlessly progressive, with death consequent to brainstem involvement or mass effect with herniation. Peripheral nervous system involvement may also occur in this variant of MS. Pathologically, the lesions are more destructive than typical MS and characterized by a large confluent area of white matter destruction with massive macrophage infiltration, pronounced acute axonal injury, and frank tissue necrosis (Figure 12). Uncommonly, multiple small lesions may be disseminated throughout the brain and spinal cord, or may coalesce to form large confluent areas of destruction. In some cases, areas of remyelination are observed. An autopsied case of Marburg disease documented pronounced post-translational changes, in which MBP was converted to an extensively citrullinated and poorly phosphorylated immature form, thought to render myelin more susceptible to breakdown, and suggesting an association with immature MBP (139). More recent neuropathological studies demonstrate that these fulminant destructive lesions are accompanied by the deposition of immunoglobulins (mainly IgG) and complement activation (30,140).

**Balo Concentric Sclerosis (BCS)**

Considered a variant of inflammatory demyelinating disease closely related to MS, BCS is characterized pathologically by large demyelinating lesions with a peculiar pattern of alternating layers of preserved and destroyed myelin, mimicking the rings of a tree trunk (Figure 12). Clinically, BCS resembles Marburg MS with similar acute fulminant onset followed by rapid progression to major disability and death within months (138,141,142). Of interest, one of the cases in Marburg’s original series (case #3) contained extensive concentric lesions (138). Reports of less fulminant disease have been described (143,144), and smaller concentric rims of demyelination have been observed in lesions from some MS patients with a more classical acute or chronic disease course. T2-weighted MR images may reveal a distinct pattern of hypo-/isointense and hyperintense rings corresponding to bands of preserved and destroyed myelin and permit ante mortem diagnosis (143,145–150).

The etiology of the concentric demyelination in this variant of MS is unknown. Pathological evaluation of 12 autopsied patients with Balo-type concentric lesions demonstrated expression of i-NOS in macrophages and microglia in all active concentric lesions. A role for hypoxia in mediating tissue injury and contributing to lesion concentricity in BCS was suggested by the expressions of HIF-1α and heat shock protein 70 (hsp70) mainly in oligodendrocytes, and to a lesser extent in astrocytes and macrophages at the edge of active lesions and in the outermost layer of preserved myelin (151). Due to their neuroprotective effects, the rim of periplaque tissue expressing these proteins may be resistant to further hypoxia-like injury in an expanding lesion, and therefore remain as a rim of preserved myelinated tissue.
Figure 12 (See color insert.) Spectrum of inflammatory demyelinating diseases. Marburg type multiple sclerosis: Macroscopically, confluent lesions lead to mass effect and herniation (A and B). Microscopically, there is extensive demyelination and axonal loss (C, LFB/PAS; D, Bielschowsky). Balo concentric sclerosis: Note the characteristic alternating bands of demyelination and preserved myelin (E). ADEM: The lesions are characterized by perivascular inflammation and only minimal, mainly perivenular demyelination (circles, F). Tumefactive lesion: Note with severe edema and mass effect (G). Creutzfeld–Peters cell: The presence of these cells should prompt consideration of an active demyelinating lesion (H). Abbreviations: LFB/PAS, luxol fast blue/periodic acid schiff; ADEM, acute disseminated encephalomyelitis. Source: From Ref. 163.
Perivenous Encephalomyelitis

Included under the term perivenous encephalomyelitis are several disorders, including ADEM, postinfectious encephalomyelitis, postvaccinial encephalomyelitis, and the most severe hyperacute variant, acute hemorrhagic leukoencephalomyelitis (Hurst disease) (137,152).

ADEM is generally a rare, monophasic, typically nonfatal disorder, most often associated with antecedent illness and is a nonspecific respiratory infection. Some fatal cases have followed measles and smallpox vaccination. In autopsied cases, measles is the most common antecedent illness, followed by rubella, mumps, varicella, and vaccinia. Other mostly nonfatal cases have followed influenza, mycoplasma, infectious mononucleosis, immunization, and the administration of antisera and some drugs. In fatal cases, the brain is grossly swollen, congested, and may show herniation, and on section may display little apart from swelling and, in some, numerous petechial hemorrhages. Microscopically, widely disseminated, small perivenous lesions are distributed throughout cerebral hemispheres, brain stem, cerebellum, and spinal cord. The lesions are of similar histological age and widely present in white matter, but also involve the deeper cortical layers, and deep gray matter structures. A distinctive feature is the presence of long sleeves of perivenous demyelination surrounded by infiltrates of reactive microglia, within which axons, while preserved relative to myelin, show tortuous and swollen profiles indicating axonal interruption (Figure 12). Narrow zones of subpial (“marginal”) demyelination in spinal cord and brain stem (along the anterior median fissure and laterally dorsal to the anterior horns), and rarely in cerebral and cerebellar cortices may be present. An accompanying mild lymphocytic meningitis is invariably present.

Attempts to recover virus from the brain, or to demonstrate viral antigens or viral nucleic acid in ADEM have been negative. The absence of typical viral-associated pathological changes argues against direct invasion of the nervous system by virus as the cause of the disease, while a latent interval between infection or immunization and the onset of the neurological illness and the presence of pathological changes similar to those in acute EAE induced by immunization with white matter or myelin support an autoimmune etiology.

AHL is usually fatal disorder characterized clinically by an abrupt onset of fever, neck stiffness, hemiplegia or other focal signs, seizures, and impaired consciousness. Thankfully, it is rare but has been reported in patients of all ages. Most cases follow an upper respiratory tract infection, typically 1 to 13 days earlier. At autopsy, the brain is congested and swollen, sometimes asymmetrically, and herniation is frequent. Multiple petechial hemorrhages are distributed diffusely throughout the brain. The perivascular lesions chiefly consist of ball or ring hemorrhages surrounding necrotic venules, sometimes with fibrinous exudates present within the vessel wall or extending into adjacent tissue. Perivascular cuffs of mononuclear cells with neutrophils are often prominent. Perivenous demyelinating lesions, identical to those of ADEM, may be present. Most authors believe that AHL represents a hyperacute form of ADEM.

Relationship of ADEM to MS

A central issue to understand the pathogenesis of MS is its relationship to ADEM. It is important to reliably distinguish MS from ADEM in order to initiate appropriate long-term therapy, but they share considerable overlap in their epidemiologic, clinical, CSF, imaging, and pathologic features. This often makes reliably distinguishing
between the two difficult when encountering patients with a single demyelinating event. ADEM is likely over diagnosed in clinical practice, and cases of “relapsing” ADEM lack pathological verification. Pathologically, the limited extent and pattern of demyelination help distinguish ADEM from MS lesions. However, the existence of “transitional” cases with features of both MS and ADEM provide some support for a shared pathogenic relationship.

**Tumefactive MS**

MS may occasionally be present as a mass lesion indistinguishable clinically and radiographically from a brain tumor (153). Patients may present with headache, aphasia, disturbance in consciousness, or seizures. Neuroimaging often reveals unifocal or multifocal enhancing lesions with associated mass effect and edema. With neuroimaging, the presence of open-ring enhancement toward the cortical surface is more likely associated with demyelinating lesions (Figure 12) (154). These patients pose considerable diagnostic difficulty and often require brain biopsy to confirm a diagnosis. Pathologically, the biopsy specimen may be mistaken for a neoplasm given the hyper cellular nature of these lesions, and the association with bizarre astrocytic forms (i.e., Creutzfeld–Peters cells) limited necrosis (Figure 12). These features are a potential trap for the pathologist, and such cases are common causes of medicolegal litigation. The histological features detailed above (intimate admixture of macrophages and reactive astrocytes, discrete borders of myelin loss, and relative axonal preservation) should confirm the diagnosis of an inflammatory demyelinating disease.

Clinical follow-up has revealed that some of these patients will develop typical MS, whereas others will have recurring tumor-like lesions. Although some cases behave like the acute Marburg variant or have features suggestive of Balo’s concentric sclerosis, there are other examples in which the course is monophasic and self-limited. Nonetheless, it is important for the neurologist to recognize that MS may present with clinical, radiographic, and even pathologic features suggestive of a primary brain malignancy.

**Neuromyelitis Optica (Devic Disease)**

NMO is an idiopathic inflammatory CNS demyelinating disease characterized by either monophasic or relapsing attacks of optic neuritis and myelitis. Pathologically, NMO lesions demonstrate extensive demyelination across multiple spinal cord levels, associated with necrosis and cavitation, as well as acute axonal damage in both gray and white matter. There is a pronounced loss of oligodendrocytes within the lesions, and inflammatory infiltrates are comprised of large numbers of macrophages associated with large numbers of perivascular granulocytes and eosinophils, as well as rare CD3+ and CD8+ T-cells. A pronounced vasculocentric deposition of immunoglobulin and complement C9 neo antigen is associated with prominent vascular fibrosis and hyalinization in both active and inactive lesions (Figure 13) (155). These findings implicate a potential role for specific autoantibody and local activation of complement in this disorder’s pathogenesis. This hypothesis is supported by serologic and clinical evidence of B-cell autoimmunity in a high proportion of patients with NMO (156).

The relationship between NMO to MS is controversial. A recently identified NMO-IgG specific marker autoantibody has been identified, which binds at or near the blood–brain barrier and outlines CNS microvessels, pia, subpia, and Virchow-Robin space, partially colocalizes with laminin and distinguishes neuromyelitis
optima from MS (157). The staining pattern of patients’ serum IgG binding to mouse spinal cord is remarkably similar to the vasculocentric pattern of immunoglobulin and complement deposition. Sensitivity and specificity for this autoantibody are 73% (95% CI = 60–86%) and 91% (95% CI = 79–100%) for neuromyelitis optica in North American patients, and 58% (95% CI = 30–86%) and 100% for opticospinal MS in Japanese patients (157). NMO-IgG binds selectively to aquaporin-4, the predominant water channel protein in the CNS (158).

WHAT DO NEW MOLECULAR STUDIES TELL US ABOUT THE MS LESION?

Genomic Approaches

Although immunocytochemistry and in situ hybridization have provided important clues to the pathogenesis of the MS lesion, these techniques are largely qualitative and static, and as such are limited in their ability to provide dynamic information on disease evolution and cellular interactions. More recently, DNA microarray technology and high throughput sequencing of cDNA have been applied to the analysis of MS brain tissue, in an attempt to identify genes that contribute to lesion pathology. These approaches allow for the simultaneous measurement of expression of thousands of genes and the identification of gene activation patterns in tissue at specific time
points. Several studies have analyzed gene expression in postmortem MS brain tissue compared to non-MS control tissue. Whitney et al. (159) did one of the earliest studies using custom printed microarrays, and compared gene expression in normal white matter with that in acute lesions in brain tissue from one MS patient. They identified many genes that were either up- or downregulated in MS plaques. Arachidonate 5-lipoxygenase, a key enzyme in the biosynthetic pathway of leukotrienes, is overexpressed in MS lesions, but since it is also expressed in other CNS diseases associated with macrophages and monocyte activation, its pathogenic relevance to MS is still uncertain. A study by Lock et al. (160) using Affymetrix GeneChip microarrays to study acute active MS lesions and chronic silent MS lesions revealed granulocyte colony-stimulating factor was highly expressed in acute lesions, and not in silent lesions, whereas transcripts encoding the IgG Fc receptor I were found over-expressed in silent lesions. These two candidate target genes were further studied in the EAE model. EAE was associated with less severe acute disease and absent chronic disease in mice deficient for the immunoglobulin Fc–receptor, whereas granulocyte-colony stimulating factor decreased the severity of early EAE. Another study revealed that MS lesions are associated with the upregulation of several known immune-related genes, as well as unique transcripts which may be relevant to MS pathogenesis (161). Comparative analysis of differential gene expression of chronic active and inactive lesions revealed significant differences in the transcriptional profiles of these two lesions, both within the lesion center and the lesion margin. Active lesion margins and centers showed upregulation of genes associated with inflammation (e.g., TNF, IL-6), compared to an under-representation in inactive lesions. Inactive lesions, however, contained many apoptosis-related genes such as bcl-x, growth factor receptor-bound protein 2, and stress-induced proteins such as hsp90A and hsp60.

Studies utilizing large-scale sequence analysis of cDNA libraries, generated from brain tissue of MS patients, have identified a number of cDNAs that are over-represented in MS, compared to those from control brain tissue (162). Among these was osteopontin (OPN), a cytokine with pleiotropic functions, including a role in inflammation and immunity to infectious diseases. Immunocytochemistry has revealed expression of OPN adjacent to both MS and EAE lesions. Since the induction and severity of EAE, and the expression of inflammatory cytokines by T-cells were greatly reduced in mice lacking the OPN gene, OPN may be a good target for future anti-inflammatory therapy.

Although gene profiling of tissue samples using microchip arrays seems powerful, easy, and plausible, there are a number of limitations that may contribute to a lack of reproducibility in published data. A primary issue relates to the quality of the sampled material, and although fresh frozen unfixed tissue is most suitable for mRNA analysis, this is rare in most brain banks, and requires careful preservation by snap-freezing. Even under the best circumstances, the structural integrity of the tissue may be impaired, leading to problems in both classifying and characterizing the sample. The nonspecific binding of antibodies to frozen tissue further limits the ability to reliably characterize the lesions. Although formaldehyde-fixed, paraffin-embedded tissue is abundant in archival brain banks, the fixation and post-mortem delay can impact both the success and quality of immunocytochemistry.

Another limitation relates to the complexity of the sampled material. Since the MS lesion results from multiple inflammatory, degenerative, and reparative events typically occurring simultaneously within the acute lesion, it is difficult to relate changes in transcriptional patterns to individual cell components. Laser capture microdissection of single cells from a stained microscopic tissue slide offers a potential...
solution. Single-cell microdissection produces highly specific results, but the amount of mRNA/cDNA obtained is minute, and usually insufficient to be combined with microarray analyses. Furthermore, the quality of extracted mRNA is a factor limiting microdissection of human material. mRNA is often degraded in human postmortem material to a degree that excludes polymerase chain reaction amplification and scanning of the transcriptome.

Another limitation is the information technology resources required to store the enormous amount of information generated, as well as to analyze and discriminate the individual signals from noise, in order to integrate the results into coherent gene clusters. Once a candidate gene is identified, its relevance must be examined by more conventional biological testing in the postgenomic phase. The identification of additional genes requires verification of the candidate in tissue samples using real-time polymerase chain reaction, in situ hybridization, or immunocytochemistry, as well as cell culture studies, transfection experiments, or the construction of transgenic mice.

Proteomic Approaches

Although genetic microarray studies may provide potential pathogenic clues to understand MS, not every transcription event leads to equally efficient protein synthesis. To understand the role of post-translational protein modification, direct information on the protein composition of cells is needed. Recent progress in protein isolation and sequence determination currently allows resolution to roughly 1000 single spots, in patterns that are highly reproducible. Mass spectroscopy identifies protein sequences of lengths, which allow determination of the protein by screening suitable databases. As with microarray approaches, proteomic studies face similar limitations relating to the quality and characterization of the starting material. Furthermore, combining protein scanning with single cell or subcellular technologies remains a methodological challenge. The use of these approaches to study MS are still in their infancy.

CONCLUSION

Neuropathological studies of MS have relied on traditional approaches such as immunocytochemistry and in situ hybridization to characterize the MS lesion. In this postgenomic era, the emergence of novel genomic and proteomic approaches continues to evolve. However, ultimately assessing the pathogenic relevance of these molecular studies will require careful correlation with classical, neuropathological findings.

REFERENCES


Clinical Features

Aaron E. Miller
Mount Sinai School of Medicine, New York, New York, U.S.A.

INTRODUCTION

Multiple sclerosis (MS) is a disease of the central nervous system (CNS) white matter that causes clinical symptoms and signs by eliciting inflammation, local edema, and demyelination in the brain, spinal cord, and optic nerves. The disease afflicts persons almost worldwide, although, with considerable epidemiological variation in incidence and prevalence rates. Women are more often affected than men, with ratios varying from 3:2 to 2:1 in various series (1,2). Young adults most frequently develop MS, but the disease may become evident at virtually any age.

The course of MS is highly variable. When disability results, it is most often a consequence of gait disturbance, impaired sphincteric function, and fatigue. The potential influence of a variety of factors on the natural history of the illness is discussed in this chapter.

DIAGNOSIS

The basis for the diagnosis of MS stems from the seminal clinicopathological observations of Charcot (3) and requires the demonstration of lesions disseminated over time and involving multiple, discrete anatomical loci in the white matter of the CNS. Schumacher et al. (4) proposed perhaps the first widely used scheme for the clinical diagnosis of MS in 1965, and all subsequent criteria have been based on these fundamental principles. Schumacher categorized patients as “clinically definite, probable, or possible” MS, according to the number of the following criteria that were satisfied:

1. Age of onset between 10 and 50 years.
2. Objective neurological signs present on examination.
3. Neurological symptoms and signs indicative of CNS white matter disease.
4. Dissemination in time: (a) two or more attacks (lasting at least 24 hours) and separated by at least a month. (An attack is defined as the appearance of new symptoms, signs, or worsening of previous ones.) or (b) progression of symptoms and signs for at least six months.
5. Dissemination in space: two or more noncontiguous anatomical areas involved.

6. No alternative clinical explanation.

Patients were classified as “clinically definite MS” if they met five or six criteria, always including the last criterion. Patients who satisfied fewer criteria were categorized as probable or possible MS.

The Schumacher criteria depended solely on clinical history and examination for diagnosis. However, in the 1970s and 1980s, technological advances permitted the demonstration of lesions that were clinically undetectable. Computer application allowed the development of evoked-response testing, a measure of electrophysiological dysfunction in the visual, brainstem auditory, or somatosensory pathways. In each of these domains, a repetitive stimulus is applied and computed elimination of random background activity allows the appearance of an identifiable waveform time-locked to the stimulus. Analysis of these responses permits identification of lesions that are not apparent clinically.

Beginning with computed tomography (CT) in the early 1970s, revolutionary advances in neuroimaging have occurred. Magnetic resonance imaging (MRI) is a much more sensitive technique for the detection of MS lesions and is usually the imaging procedure of choice. These new imaging modalities have provided a means of demonstrating anatomical lesions that are not clinically evident (see Chapter 7). Indeed, cranial MRI demonstrates activity, as much as five to ten times, more frequently than clinical relapse is apparent (5). Still newer MR techniques, such as measurements of brain atrophy, MR spectroscopy (MRS), magnetization transfer, and diffusion tensor imaging promise further progress in our assessment and understanding of MS through neuroimaging.

In addition, increasingly sensitive methods for the study of cerebrospinal fluid (CSF) led to the recognition that most MS patients have evidence of abnormal immunoreactivity that can be demonstrated by CSF analysis. Abnormalities include elevated immunoreactivity, elevated immunoglobulin IgG levels (6,7), increased IgG index (8), increased IgG synthesis rate (9), and oligoclonal bands (10–13).

The opportunities created by these new techniques led to the development of new criteria by a committee chaired by Poser (14). The Poser criteria (Table 1) modified Schumacher’s by allowing the demonstration of “paraclinical” lesions (i.e., lesions detected by evoked-response testing or neuroimaging studies). In addition, the new criteria established an additional category of laboratory-supported MS based on the inclusion of CSF abnormalities.

The Poser criteria proved a useful tool for the diagnosis of MS for two decades, despite their having been developed primarily to classify the disease for research purposes. However, the Poser criteria were developed when the use of MRI was in its infancy. This imaging modality has clarified much about the biology of MS and a reassessment of the criteria used to diagnose the disease in the context of such knowledge was warranted. This had become critically important because of the availability of new drug therapies (see Chapters 14, 15). Because it appears beneficial to initiate treatment early, before permanent neurologic deficits develop, it is important to be able to diagnose the disease with reasonable certainty in the initial stages of the illness.

Because of the advances in understanding achieved through MRI and differences in the categorization of MS subtypes, an International Panel, under the chairmanship of W. I. McDonald gathered in 2000 and published its recommendations the following year (15).
The McDonald committee sought to devise criteria that
1. could be used by practicing physicians,
2. could be adapted for clinical trials,
3. would integrate MRI into the diagnostic scheme,
4. would include a scheme for the diagnosis of primary progressive MS, (a category that was not recognized at the time the Poser committee met),
5. would clarify certain definitions,
6. would simplify diagnostic classifications and descriptions, and
7. would retain as many as possible useful features of the existing criteria.

The committee emphasized several general conclusions:

1. Definitive diagnosis requires objective evidence of dissemination in time and space, as well as the exclusion of other, better explanations for the clinical picture.
2. Definitive diagnosis requires that clinical evidence depend primarily on objectively determined clinical signs. Historical accounts of symptoms are alone insufficient for diagnosis of MS.
3. Radiologic and laboratory investigations may add to a clinical diagnosis and may be essential in making a diagnosis when clinical presentation alone does not allow diagnosis.
4. Following diagnostic evaluation, an individual is usually classified as either having MS or not having MS.

These first two principles re-emphasized those established previously. However, the McDonald criteria eliminated the Poser category of “probable MS.” In the new diagnostic scheme, all patients who neither meet criteria for “definite” MS nor have a specific alternative diagnosis established are regarded to have “possible” MS.

### Table 1 Poser Criteria for Multiple Sclerosis

<table>
<thead>
<tr>
<th>Category</th>
<th>Clinical attacks</th>
<th>Paraclinical evidence</th>
<th>CSF&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinically definite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDMS A1</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>CDMS A2</td>
<td>2</td>
<td>1 and</td>
<td>1</td>
</tr>
<tr>
<td>Laboratory-supported definite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSDMS B1</td>
<td>2</td>
<td>1 or</td>
<td>1 +</td>
</tr>
<tr>
<td>LSDMS B2</td>
<td>1</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>LSDMS B3</td>
<td>1</td>
<td>1 and</td>
<td>1 +</td>
</tr>
<tr>
<td>Clinically probable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPMS C1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CPMS C2</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>CPMS C3</td>
<td>1</td>
<td>1 and</td>
<td>1</td>
</tr>
<tr>
<td>Laboratory-supported probable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSPMS D1</td>
<td>2</td>
<td>2</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup>OB/IgG, oligoclonal bands or increased IgG.

*Abbreviations:* CSF, cerebrospinal fluid; CDMS, clinically definite MS; LSDMS, laboratory supported definite MS; CPMS, clinically probable MS; LSPMS, laboratory supported probable MS.
The committee also clarified several critical definitions to facilitate diagnosis:

1. “An ‘attack’ refers to an episode of neurological disturbance of the kind seen in MS, when clinicopathological studies have established that the causative lesions are inflammatory and demyelinating in nature.”
   
   A. An attack, defined either by subjective report or by objective observation, should last for at least 24 hours.
   B. “Whereas suspicion of an attack may be provided by subjective historical reports from the patient, objective clinical findings of a lesion are required to make a diagnosis of MS.”
   C. “Single paroxysmal episodes (e.g., a tonic spasm) do not constitute a relapse, but multiple episodes occurring over not less than 24 hours do.”

2. A new attack is defined as symptoms beginning 30 days after the onset of the previous attack.

3. “Abnormality” on MRI required the more stringent criteria of Barkhof et al. (16) and Tintore et al. (17), rather than the more sensitive but less specific criteria of other authors, including Paty et al. (18) and Offenbacher et al. (19). The more stringent criteria require three out of four of the following:
   
   A. One gadolinium-enhancing lesion or nine or more lesions on T2-weighted images.
   B. One or more infratentorial lesion.
   C. One or more juxtacortical (involving subcortical U fibers) lesion.
   D. Three or more periventricular lesions.

   Only lesions that are at least 3 mm in cross-section are counted. The panel also allowed the substitution of a spinal cord lesion for a brain lesion.

   The committee further commented, “Whereas it is possible that, in the absence of brain lesions, two or more spinal cord lesions clearly separated in time and/or space could satisfy criteria, prospective data in this regard are still awaited.”

4. “Abnormality on CSF analysis can provide supportive evidence of the immune and inflammatory nature of lesion(s), which may be helpful when imaging criteria fall short, when they lack specificity (as in the older patient), or when the clinical presentation is atypical.” CSF determinants are:
   
   A. Presence of oligoclonal bands different from those in serum and preferentially obtained by isoelectric focusing.
   B. Presence of increased IgG index.
   C. Lymphocytic pleocytosis should not exceed 50 cells.

5. Abnormal visual-evoked potential should be typical of MS, i.e., delayed but with a well-preserved waveform.

6. MRI criteria for dissemination of lesions in time:
   
   A. If the first scan was obtained three or more months after the onset of symptoms:
      a. A gadolinium-enhancing lesion satisfies dissemination in time.
b. If no gadolinium-enhancing (Gad+) lesion, a follow-up scan (three months recommended) with either a new T2 or Gad+ lesion satisfies dissemination in time.

B. If the first scan was obtained less than three months after the onset of the clinical event, a second scan should be obtained three or more months after the onset.

a. A new Gad+ lesion equals dissemination in time.

b. If no Gad+ lesion, do another scan, “not less than three months after the first scan,” either a new T2 or Gad+ lesion equals dissemination in time.

Using the above definitions, the diagnostic scheme for determination of clinically definite multiple sclerosis (CDMS), i.e., the satisfaction of the criteria of dissemination in time and space, is shown in Table 2.

The criteria for the diagnosis of primary progressive multiple sclerosis (PPMS), which are derived from a proposal by Thompson et al. (20), have not been established on the basis of the type of prospective MRI analysis used for the criteria of dissemination in space recommended for relapsing MS. The necessity of a positive CSF for establishing the diagnosis is also uncertain. The importance of this information may become clearer after further analysis of the patients included in a recently concluded trial of glatiramer acetate versus placebo in PPMS, which included patients both with and without positive CSF.

The diagnostic criteria established by the McDonald committee are clearly an important advance, especially by incorporating MRI evidence. Nonetheless, they

### Table 2 New Multiple Sclerosis Diagnostic Criteria

<table>
<thead>
<tr>
<th>Clinical (attacks)</th>
<th>Objective lesions</th>
<th>Additional requirements to make diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 or more</td>
<td>2 or more</td>
<td>None: clinical evidence will suffice (additional evidence desirable but must be consistent with MS)</td>
</tr>
<tr>
<td>2 or more</td>
<td>1</td>
<td>Dissemination in space by MRI or positive CSF and 2 or more MRI lesions consistent with MS or further clinical attack involving different site</td>
</tr>
<tr>
<td>1</td>
<td>2 or more</td>
<td>Dissemination in time by MRI or second clinical attack</td>
</tr>
<tr>
<td>1 mono-symptomatic</td>
<td>1</td>
<td>Dissemination in space by MRI or positive CSF and 2 or more MRI lesions consistent with MS Dissemination in time by MRI or second clinical attack</td>
</tr>
<tr>
<td>0 (progression from onset)</td>
<td>1</td>
<td>Positive CSF Dissemination in space by MRI evidence of 9 or more T2 brain lesions 2 or more cord lesions or 4–8 brain and 1 cord lesion Positive VEP with 4–8 MRI lesions Positive VEP with less than 4 brain lesions plus 1 cord lesion Dissemination in time by MRI or continued progression for 1 yr</td>
</tr>
</tbody>
</table>

*Abbreviations:* MS, multiple sclerosis; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid.
leave a number of ambiguities and have been criticized by some for the stringency of the MRI criteria (21). Undoubtedly, further revisions and refinements of the diagnostic process will be forthcoming.

Nonetheless, the use of McDonald criteria has allowed earlier diagnosis of MS than the Poser scheme did. In one study, using the McDonald criteria allowed 38/79 (48%) patients to be classified as “definite” MS compared to 16/79 patients by (20%) applying the Poser criteria, after one year of follow-up (22). In another similar study, 51/139 (37%) met McDonald criteria for “definite” MS, whereas only 15/139 (11%) did so using the Poser requirements (23).

Physicians should recognize the difference between making a definitive diagnosis of MS and making a decision to initiate therapy. Treatment decisions should be based on the clinician’s risk-potential benefit analysis (and sometimes cost-potential benefit analysis) and discussion with the patient about the pros and cons of initiating therapy. Therapy might be recommended, even in the absence of a definitive diagnosis, if the physician judges that the risk-potential benefit ratio of starting treatment exceeds that of deferring treatment.

AGE OF ONSET

Although almost all MS patients develop their initial symptoms during young to middle adulthood, the disease can occur at virtually any age. Convincing cases in childhood have been reported as early as 15 months (24–26), with onset younger than age 10 occurring in about 0.3% of cases (27–29). The early-onset disease does not appear to differ clinically from that starting late, although there may be an even greater female preponderance in this age group (30).

Onset of the disease after the age of 50 has been considered rare, although a more recent report suggests that MS may be more common than suspected in older individuals (31). New techniques facilitating diagnosis are contributing to increased recognition of cases in this age population. When the disease begins after the age of 40, it tends more often to follow a progressive course, particularly characterized by worsening spastic paraparesis (32–35).

CLINICAL MANIFESTATIONS

Motor Symptoms

Corticospinal tract involvement occurs with the initial attack of MS in 32% to 41% of patients (36–39), but it is present to a significant degree in 62% (37,39) of patients with chronic disease. Symptoms may also include “heaviness,” “stiffness,” or even, pain in an extremity. The legs are much more frequently involved than the arms; when both are involved, symptoms in the legs usually appear earlier. Involvement often begins with one leg, but in most patients, both are eventually affected, although the severity may be quite asymmetric. Signs of corticospinal tract pathology may be as minimal as abnormal reflex activity, but the disease frequently progresses to severe spastic paraparesis. Hyperactivity of the deep tendon reflexes, often including crossed tibioadductor and puboadductor reflexes, is seen in most patients. Clonus, which may be sustained and severe, is often present. This is most common at the ankle, but it may be found at other
sites as well. The Babinski reflex is frequently present, at times as the only manifestation of corticospinal tract dysfunction.

Spasticity is very commonly seen in the legs, but may also occur in the arms. At times this abnormality actually helps a paretic patient walk (40,41), but in other patients it may produce discomfort or pain, cause flexor or extensor spasm (42,43), or interfere with personal hygiene (adductor spasticity). In addition, spasticity may result in disturbed sleep or interference with sitting. If inadequately addressed and treated, it may ultimately result in irreversible joint contractures. Patients themselves complain of stiffness, cramps, spasm, or pain. In the upper extremity, weakness often predominates in the distal musculature. At times, this is accompanied by extensive atrophy, presumably reflecting demyelination in the root entry zones of the spinal cord (36).

Corticospinal tract involvement most often results from demyelination within the spinal cord, although other sites, including the medullary pyramids, basis pontis, cerebral peduncles, and deep hemispheric white matter, may also be involved (44). A hemiparetic pattern of motor weakness may be seen, but is uncommon. Indeed, on occasion MS may present with the acute onset of hemiparesis including the face (45) and may clinically look like lacunar infarction. Despite an initial appearance suggesting a vascular etiology, MRI may lead to the diagnosis of MS, with lesions most often evident in the posterior limb of the internal capsule. Subtle abnormalities of bimanual coordination have recently been reported in MS, presumably from involvement of the corpus callosum (46).

**Somatosensory Symptoms**

Sensory complaints are frequent among the earliest symptoms of MS with a recent survey indicating that they were the first manifestation in 43% of patients, but this figure may have included visual as well as somatosensory phenomena (47).

The symptoms are often perplexing for the clinician, especially during the onset bout, because they are frequently unassociated with objective signs on the neurological examination. In addition, the anatomical distribution is often peculiar, not corresponding to recognized dermatomal, peripheral nerve, or homuncular patterns. Patients usually complain of numbness, but more often are referring to a subjective positive sensation than to diminished or absent sensation. Common complaints include tingling, burning, tightness, a feeling like “procaine (Novocaine) wearing off,” or a sensation that a garment, such as a glove or a girdle, is being worn. Often the abnormal sensation occurs in a band-like fashion around a limb or the abdomen. Sometimes only a patch of abnormal sensation is reported.

These complaints likely reflect lesions of the myelinated posterior columns (fasciculi gracilis and cuneatus), rather than the spinothalamic tracts (36). In contrast, objective sensory signs of diminished pain and temperature sensation indicate involvement of the latter pathways. Vibratory sense impairment is extremely common and almost always precedes detectable abnormality of joint position sense. The author has observed subtle reduction in the ability to perceive a vibrating tuning fork in many mildly affected patients early in the course of the disease. Reduced perception of pinprick or temperature sensation is less frequently seen. It, too, has a variable pattern of distribution, but may show a spinal cord level. A picture resembling Brown-Sequard syndrome is occasionally seen (48).

A fairly specific sensory symptom of MS is Lhermitte’s sign (a misnomer, since this is a subjective complaint) (49,50). Patients complain of sudden electric-like
sensations radiating down the spine or extremities for a brief moment. This typically occurs when flexing the neck.

In a recent survey, 67% of patients with MS reported pain at some point in their disease course, a frequency comparable to that of controls (47). However, twice as many patients with MS reported active pain than did the control group. They also tended to have pain most often in the extremities and trunk, whereas the controls more often reported head, back, and neck pain. Several distinct pain syndromes may occur in MS patients. Some experience severe, lancinating neuralgic pains in the limbs or elsewhere; others complain of more persistent, intolerable dysesthesias, frequently with a burning quality (42,43). Patients with spasticity often report painful spasms or cramping sensations in the legs.

Although low-back pain is a very common ailment among the general population, it is perhaps even more among persons with MS. This may be related to abnormal postures and gaits associated with weakness and spasticity. Radicular pain may occur occasionally in the absence of compressive pathology and, in one report, was the presenting complaint in 3.9% of patients with newly diagnosed MS (51).

Osteoporosis is another concern for patients with MS and another source of pain. Cosman et al. (52) reported a history of fractures in the absence of major trauma in 22% of MS patients compared with only 2% of controls ($P < 0.002$). Determining bone mass by dual X-ray absoriometry, the authors observed that over two years of prospective follow-up both women and men with MS lost substantially more bones than controls. There was a trend, which did not reach statistical significance, for diminished ambulatory status and long duration of steroid therapy to predict higher bone loss. In another study, however, this group noted that “after controlling for age, cumulative steroid use was not a determinant of bone mineral density,” (53) a finding of Schwid et al. (54). However, low vitamin D intake and diminished exposure to sunlight appear to be major contributors to the problem.

Although headache has not been particularly associated with MS, one report cited a patient with severe acute headache, associated with a solitary new lesion in the periaqueductal gray region (55). This unusual case supports observations in patients with implanted electrodes, in which perturbation in this area can produce headache.

In another unusual case, headache, mimicking subarachnoid hemorrhage occurred. A patient with a history of facial myokymia developed apoplectic headache and a third nerve palsy. Investigations revealed no evidence of subarachnoid hemorrhage or aneurysm, but MRI showed more than 30 white matter lesions, and CSF examination revealed oligoclonal bands (56).

### Brain Stem Symptoms

Although protean manifestations of abnormal brain stem function appear in MS, impairments of ocular motility are most frequently encountered. Nystagmus, most often horizontal, occurs commonly with a frequency as high as 40% to 70% in some series (36,37). Other forms of nystagmus, including rotatory, upbeat, and downbeating, occur less often. In most patients the nystagmus is asymptomatic, but other patients may complain of blurred vision, images jumping (oscillopsia), or sometimes double vision.

Internuclear ophthalmoplegia, either unilateral or bilateral, is a common manifestation of MS, resulting from lesion(s) of the median longitudinal fasciculus (57,58). In young adults, this sign, in fully developed form, consists of failure of
the eye ipsilateral to the lesion to adduct, whereas the contralateral eye abducts fully, but with horizontal nystagmus ["dissociated nystagmus" (58)]. The eyes are orthophoric at rest and patients do not usually complain of visual symptoms. Incomplete forms are common (59,60).

Other abnormalities of extraocular motility, including horizontal (sometimes bilateral) (61) and vertical gaze paresis (62,63), the one-and-a-half syndrome (64,65), dysfunction of individual nerves to the extraocular muscles (36), and skew deviation (66), may occur. Additional unusual eye movement abnormalities including ocular contrapulsion (67), acquired convergence-evoked pendular nystagmus (68), and divergence insufficiency (69) have recently been described. Transient or sustained diplopia is a common complaint (36–39). Alternatively, patients with prominent nystagmus may report that images are jumpy or jittery, a symptom known as oscillopsia.

Dysarthria is frequent in MS, especially in chronic, more advanced cases. A particular type of speech disturbance, known as “scanning speech,” has long been considered most typical of MS. However, impaired articulatory agility is much more common (70). Scanning speech refers to a particular rhythm and cadence in which each word or syllable is given emphasis. "There is a pause after every syllable and the syllables themselves are pronounced slowly" (3). However, scanning speech seldom interferes with communication.

Other types of dysarthria occur less frequently. Nasal speech may result from involvement of cranial nerves IX and X. Bilateral involvement of corticobulbar tracts may lead to the explosive, poorly modulated speech characteristic of pseudobulbar palsy (70). Two patients have been reported in whom a lifelong problem with stuttering disappeared with the onset of signs of MS (71).

Although facial paresis is usually of the central type and is seen with other motor signs, a facial palsy of peripheral type develops occasionally (72). This presumably results from demyelination of the facial nerve within the brain stem itself.

Blepharospasm, generally in association with other brain stem signs, has been described (73). Facial myokymia an "undulating, wavelike fascicular twitching," usually beginning in the orbicularis oculi, occurs occasionally (74,75).

Auditory disturbance is uncommon in MS, although hearing loss, either unilateral or bilateral, sometimes occurs (76). It is generally caused by lesions in the brain stem (77,78), but an unusual case of deafness due to cerebral disease has been reported (79). In contrast, a case of central hyperacusis has been recently described (80). Among patients presenting with isolated hearing loss, MRI frequently reveals unexpected evidence of MS (81). Tinnitus is experienced occasionally. Vertigo is a frequent complaint of MS patients, usually as part of an acute exacerbation and associated with other signs of brain stem dysfunction. Intractable hiccups have been reported as an unusual manifestation of MS (82,83).

Visual Pathway Symptoms

Optic neuritis is one of the most common manifestations of MS, occurring in 14% to 23% of cases (36–39). Patients usually complain of dimming of vision unilaterally, generally accompanied by photophobia and pain aggravated by eye movement. Examination reveals diminished visual acuity of varying severity, and detailed visual field evaluation frequently shows a central scotoma (84). Visual loss is seldom total, and good recovery of vision usually occurs within six months, even when the initial visual loss is extremely severe (85,86). Fundoscopy may
show a swollen optic nerve head with hemorrhages or exudates (papillitis), or a normal optic disk (retrobulbar neuritis).

Even in the absence of acute optic neuritis, many patients demonstrate clinical abnormalities of optic neuropathy. This may be manifest by diminished visual acuity, impairment of color vision as detected with Ishihara plates (87), abnormal visual field examination, or a positive “swinging flashlight test” (88) indicative of an afferent pupillary defect (Marcus Gunn pupil). Subtle abnormalities of vision may also be detected by the use of low-contrast sloan letter charts, even in patients with visual acuity of 20/20 (89). Fundoscopy may reveal optic atrophy. Visual-evoked responses will often be abnormal in patients who never had clinical evidence of optic nerve disease (90,91). Many patients experience bilateral optic nerve dysfunction, but blindness is relatively uncommon.

Virtually, any type of visual field defect may occur in MS depending on the site of the inflammatory-demyelinating lesions. For example, recent reports have cited the presentation of the disease with bilateral homonymous visual field defects (92,93) and acute quadrantic field loss (94). Others have reported similar deficits occurring in the course of MS and, as in the former reports, associated with anatomically appropriate lesions demonstrated by MRI.

Olfaction and Gustation

Although spontaneous complaints about abnormalities in the senses of smell and taste are infrequent (95,96), recent reports have demonstrated that disturbances of olfaction are relatively common. Two studies employing the University of Pennsylvania Smell Identification Test (UPSIT) reported abnormalities in 15% and 38.5%, respectively. In the latter study of 26 patients, Doty et al. (97) found 7.7% with severe impairment, 19.3% moderate, and 11.5% mild. They noted a strong negative correlation between a high UPSIT score (normal olfaction) and the number of lesions within the inferior frontal and temporal lobe regions, which are involved with olfaction.

Other investigators have confirmed the presence of olfactory disturbances (98,99) and also correlated diminished olfaction with lesion load in the olfactory region of the brain (99). Hawkes et al. (100) further demonstrated the involvement of olfactory systems by noting abnormalities to hydrogen sulfide evoked responses (H$_2$S-ER) in MS patients. A group with the disease had statistically increased latency and decreased amplitude in the H$_2$S-ER compared to controls. In general, results on the UPSIT correlated well with the evoked response, although an abnormality on one test did not always indicate an abnormality on the other.

Patients with MS rarely complain of disturbances in taste (101). However, a case has been reported in which hemiageusia was the presenting manifestation of the disorder, preceding other signs of trigeminal and brainstem involvement by more than a week (102). A lesion in the right medulla in the floor of the fourth ventricle was observed on MRI. A previous report had noted the occurrence of hemiageusia in a patient with simultaneous right facial numbness (103).

Cerebellar Manifestations

Disturbances resulting from both vermian and hemispheric cerebellar lesions are common in MS. Gait ataxia was an initial complaint in 13% in one series (36). Although common in patients with chronic disease, its incidence is difficult to discern
from published series because many patients are included under vague categories, such as balance. Examination may show appendicular ataxia, dysmetria, intention tremor on finger-nose-finger test, or dysdiadochokinesia when the patient tries to perform rapid, alternating movements. Dysmetria on the heel-knee-shin test is also frequently present. At times, the tremors, present even at rest, may assume violent proportions with attempted movement (so-called rubral tremor). Limb ataxia or intention tremor has been reported in 45% to 50% of patients with chronic disease (37,39).

**Cognitive and Psychiatric Disturbances**

In recent years, awareness of cognitive dysfunction in MS patients has increased, although abnormalities have been variably reported in 0% to 90% of cases (104,105). A follow-up study of 45 patients with MS, initially studied early in their disease course, found that only 20 of 37 initially cognitively normal individuals remained so after 10 years (106). Clearly, severe dementia is unusual in MS, but more subtle abnormalities of cognitive function are common. These are often unnoticed by patients, families, or physicians, but they may be detected on more formal neuropsychiatric evaluation. The most frequently encountered difficulties are with memory, attention–concentration, and conceptual reasoning–problem solving (104–109). Although cognitive impairments are variable, they typically follow patterns usually associated with subcortical lesions. Several recent studies have provided evidence of impaired driving in patients with MS and cognitive dysfunction (110–112). Aphasia, apraxia, neglect, and other cognitive features of cortical pathology are uncommon, although several cases with language impairment as the sole or major feature of an acute exacerbation have been reported. Zarei et al. (113) have recently reported six patients who presented with progressive dementia marked by prominent amnesia but also featuring classical cortical features such as dysphasia, dyslexia, and dysgraphia, in the absence of any other neurological signs. All patients had mood disturbances and three had a long history of severe depression. Eventually prominent neurologic signs with marked disability occurred in all. The authors proposed the concept of a “cortical” variant of MS, which appears consistent with recent pathologic and MRI observations suggesting that cortical involvement is more widespread than previously believed. Thus far, correlation of cognitive status with either duration or severity of illness has been poor. Dementia did not correlate with the number of distribution of lesions on MRI scans in one study (114), but a more recent study did find such a correlation (115). More focal cognitive abnormalities, such as aphasia (116–118) and neglect (119), have been reported, but they are very unusual.

Earlier literature described euphoria as a feature of MS (120). However, depression is now recognized much more commonly, with 50% or more of patients experiencing this affective disturbance in some form during the course of the illness (121–123). Although this is usually relatively mild, major depression can occur (123). Suicide may be a major cause of mortality, accounting for 15% of adult deaths in one series (124). Recently, Feinstein (125) identified warning signs that include living alone, having a family history of mental illness, and reporting social isolation. Patients with a prior history of major depression, anxiety disorder, or alcohol abuse are also particularly vulnerable. The so-called euphoria is actually the inability to inhibit emotional expression, resulting in “inappropriate” laughing and crying. This occurs with subcortical forebrain lesions (126). Other instances of apparent euphoria seem to be associated with evidence of significant cognitive decline. Euphoria is rarely, if ever, seen as an early sign in patients with mild symptoms (41).
On rare occasions, other psychotic states, mimicking schizophrenia or other delusional syndromes, may occur in MS. Limited data suggest that the patient with these symptoms may have more disease in the temporal lobe periventricular area (127,128). Also, one must always consider the possibility of iatrogenic symptomatology in patients being treated with a variety of the medications used in MS.

Fatigue and Sleep

Fatigue is one of the three most frequently disabling symptoms of MS (129) and may be considered abnormal in as many as 78% of patients (130,131). A particular feeling of enervation, severe enough to prevent a patient from carrying out duties and responsibilities or to interfere with work, family, or social life, occurs (132). This specific, but poorly understood, type of fatigue in MS must be distinguished from symptoms of depression, medication side effects, consequences of other medical conditions such as anemia, hypothyroidism, or simple physical tiredness. No definitive explanation for fatigue in MS has been established. One type of fatigue, so-called handicap fatigue, is characterized by the requirement for an increased effort to perform routine tasks. This may be a consequence of the fact that nerve conduction in demyelinated fibers is susceptible to exhaustion, rate-dependent block, and conduction block with increased temperature.

Alternatively, patients with MS may experience the so-called systemic fatigue, which they describe as chronic lack of energy, tiredness, or malaise that are often sufficiently severe to prevent activities at home or work. Similar chronic lassitude is a common feature of a number of inflammatory diseases, suggesting that it may be due to the effects of soluble immune mediators on the CNS. Objective studies have provided little support for an alternative suggestion of an autonomic basis for fatigue. The MRI abnormalities typically seen in MS do not correlate with fatigue, but some recent studies suggest that a relationship may exist between cortical function and fatigue.

The prevalence of sleep complaints was three times greater in a group of MS patients than in controls. Sleep complaints were associated with greater levels of depression. In addition, several lesion sites subserving supplementary motor areas were related to the sleep complaints. The authors speculated that such lesions might be related to the production of sleep-disturbing nocturnal spasms (133).

Bladder, Bowel, and Sexual Disturbances

Disturbances of defecation and especially micturition are among the most disabling features of MS, occurring in up to 78% during the course of the illness (134). Patients may complain of urinary frequency, urgency, and incontinence. Alternatively, the urge to urinate may be accompanied by an inability to voluntarily initiate urine flow. History alone is an unreliable indicator of the physiological status of micturition and must be supplemented by further investigation (135,136). Usually, this requires only a determination of voided volume followed by measurement of residual urine volume, either by direct catheterization or by some other method for estimation, such as ultrasonography or radionuclide study (137). Disturbances of micturition may be divided into failure to store urine, failure to empty the bladder adequately, or a combination of both. In some patients, good contraction of the bladder detrusor is inappropriately associated with contraction of the external urethral sphincter, rather than relaxation. This condition, known as detrusor-external
sphincter dyssynergia, may then lead to retention of urine and, particularly in males, to vesicoureteral reflux, with the threat of hydronephrosis and progressive renal failure (138). Retention of urine also increases the risk of urinary tract infection which, in turn, may suddenly precipitate urinary symptoms.

Bowel dysfunction in MS has received less attention than disturbances of micturition. However, studies have shown a prevalence rate of constipation ranging from 39% to 53% (139–142). The suggested causes include slow colonic transit due to autonomic dysfunction, abnormal rectal function, and intussusception (141–143). The problem is often compounded by a tendency of patients to reduce fluid intake in an attempt to decrease urinary frequency and urgency. In a recent survey of unselected outpatients, Hinds et al. (139) found that 51% of patients had experienced bowel incontinence at least once in the preceding three months, whereas 25% had experienced the symptom at least weekly. Fecal incontinence appeared to correlate with degree of disability, duration of disease, and the presence of urological symptoms.

Sexual symptoms are also common among MS patients. Men most often experience erectile dysfunction, but may also suffer from problems with ejaculation (135,144). These symptoms typically accompany abnormal micturition. Women most typically experience difficulty in achieving orgasm, but may also complain of problems with lubrication (145). Both men and women may also complain of diminished libido. In contrast, a recent case of episodic hyperlibidinism has been reported (146).

**Paroxysmal Symptoms**

Although some studies report an increased prevalence of epileptic seizures compared to the general population, a recent population-based epidemiologic report from Olmsted County, Minnesota found the occurrence to be similar in MS patients and the general population (147). Recently, five patients were described who had juxtacortical lesions in the temporal region and presented with temporal lobe epilepsy as the sole manifestation of MS (148).

In contrast to true epilepsy, many MS patients experience other types of stereotyped, repetitive paroxysmal symptoms and signs. Tonic “seizures” have been reported frequently (48,149–153). Patients suddenly experience dystonic posturing of part of the body, most typically the hand or arm, lasting 30 seconds to 2 minutes. Sometimes the attack is painful. Episodes may occur infrequently or many times a day and tend to cluster for periods of weeks to months. The anatomical lesions, apparently responsible for the dystonia, have been found in a variety of sites, including the basal ganglia, the internal capsule, the thalamus, the cerebral peduncle, and the cervical cord (154–157).

Episodes of paroxysmal dysarthria, with or without other brain stem dysfunction, have also been reported (158,159). The author has encountered a patient with episodic aphasia. Some patients experience paroxysms of itching, and this has been reported as the initial symptom of MS (160). Hemifacial spasm may occur and may be associated with lesions in or near the facial nucleus, as identified by MRI (161).

Trigeminal neuralgia is the most common paroxysmal disturbance. The clinical syndrome is usually indistinguishable from that in non-MS patients, except that onset tends to occur at an earlier age and symptoms are more frequently bilateral (162). Occasionally, the usual feature of excruciating, lancinating pain is associated with objective sensory disturbance in the MS patient.
Movement Disorders

A variety of movement disorders occasionally occur in MS. An unusual case of hemiballismus in a convincing case of infantile MS has been reported (24). Both kinesigenic dystonia (163) and paroxysmal kinesigenic choreoathetosis (164) have marked the onset of MS. Segmental myoclonus of spinal origin has been noted (165), and a rare case of trismus has been reported (166). Micrographia, a sign more typically associated with Parkinson’s disease, was reported in association with an enhancing lesion in the dominant parietal white matter (167).

Autonomic Disturbance

Although not commonly reported, autonomic dysfunction (other than disturbances of bowel and bladder) may be noted. Abnormal sweating has been described, and some patients have coldness or discoloration of the legs or feet (168,169a,169b). Autonomous respiration, a syndrome in which the patient loses voluntary control of breathing, has been described (170). Respiratory failure may rarely occur as a result of bilateral diaphragmatic paralysis with or even without significant quadriparesis (170–172).

Several patients who experienced acute relapses associated with hypothermia have been reported (173). Most of the patients were severely disabled and all displayed signs during relapse of other brainstem lesions, suggesting that the hypothermia may be secondary to brainstem, rather than hypothalamic, involvement. Thrombocytopenia commonly accompanied the disturbed temperature regulation.

COURSE

The course of MS is highly variable. Lublin and Reingold (174), after polling many MS experts, defined four temporal patterns. In most patients (80–85%), the disease initially follows a relapsing pattern of acute exacerbations (attacks) punctuated, usually after some improvement, by periods of stability (remissions). This form of disease, in which the baseline is stationary between attacks, is referred to as “relapsing–remitting MS.” Approximately, 10% to 15% of patients never manifest acute attacks. Rather, from the onset they follow a course of steady worsening, perhaps with occasional plateaus. This type is referred to as, “primary progressive MS.” A very small proportion of patients (probably fewer than 5%) start off as if they are going to have primary progressive disease. However, the course is then interrupted by discrete exacerbations. This form is thus referred to as “progressive-relapsing MS.” Of those patients presenting initially with the relapsing–remitting form of the disease, many (probably 50–60%), after some years, begin to steadily worsen between attacks. Some of them do continue to have discernible exacerbations, but the hallmark of this form of the disease, which is referred to as “secondary progressive MS,” is that the baseline does not remain stable, as the patient gradually deteriorates.

In usual cases, the initial attack of MS resolves completely, or nearly so, and the patient remains entirely well until the next discrete episode. In one series, more than 25% of patients relapsed within one year and more than 50% within three years (36). However, Muller (37) found a latent phase of at least 15 years in 6% of patients followed for that period. The attack rate varies from 0.1 to 0.85/yr in the early stages.
of the illness, according to several series (36,175–179). In several recent clinical trials that selected patients experiencing recently active disease, attack rates in placebo-treated groups ranged from 0.87 to 1.3 annually (180–182). However, in most studies, the frequency of identifiable attacks appears to diminish with increasing duration of illness (36,175,183,184).

Exacerbations most often develop over hours to days. However, the onset may at times be abrupt (strokelike) and in other cases more indolent. Remission of symptoms tends to occur within weeks to a few months. Muller (37) reported that up to 85% of patients seen within two months of relapse remitted completely, but the rate fell to 30% at three months and 10% at six months. Recent MRI studies indicate that Gad enhancement, currently the best imaging marker of new activity, usually disappears within four to six weeks (185). Once progression has begun, it generally continues, but at a relatively slow rate. Natural history studies by Weinschenker et al. (186) found that 50% of patients required assistance to walk by 15 years after the onset of symptoms. In one series of patients scored with the widely used Kurtzke expanded disability status scale (EDSS), those who were ambulatory when first examined worsened by 1 to 1.5 steps over the next five years (187). Those who required assistance to walk or were wheelchair-dependent worsened 0.3 to 0.7 steps over that period. Even in progressing patients, the disease course may appear to stabilize for long periods. In one clinical trial, approximately 20% of patients selected because of their progressive course showed little or no worsening during serial examinations over the next 12 months (188). Wynn et al. (189) reported a 74% survival rate at 25 years, compared with an expected 86%, and Kurtzke et al. (190) similarly found about 75% of normal survival in that interval. A recent Danish report noted that the excess mortality from MS has been halved over the past half century (191).

At times MS follows an extremely benign course. The patient experiences two or more attacks with complete remissions and no cumulative disability over many years (36). In Weinshenker’s cohort, 10% to 20% of MS patients remained mildly affected after 25 years (186). However, most studies show only 11% to 34% of patients are able to work within 15 years (36,178,192). A recent population-based study in Olmsted County, Minnesota, found that among 39 patients with an EDSS score $<2$, five or more years after the onset of MS, only one needed a cane after another decade (193).

Unfortunately, predicting the course of MS in an individual patient is virtually impossible. Generally, patients whose initial attacks are marked by sensory symptoms have a better prognosis than those who early manifest corticospinal tract or cerebellar dysfunction (36,194,195). The course of the disease over the first five years provides a clue, on a statistical basis, to the subsequent progression. Thus, in a group of U.S. servicemen, nearly 90% of those with minimal disability at five years after onset continue to be ambulatory at 15 years. Of those with moderate disability, but walking unaided at five years, 60% were walking independently at 15 years (196). Age of onset also influences prognosis, with patients experiencing their first symptoms after age 40 tending to follow a more rapidly progressive course.

No factors have been clearly recognized to alter the long-term course of MS. However, certain conditions may tend to precipitate acute exacerbations. Viral infection seems to trigger attacks (197,198). This may occur by stimulation of interferon gamma which, in turn, leads to increased antigen presentation precipitating the episode (198).

The role of stress, both physical and psychological, in precipitating exacerbations of MS has been controversial (199). Early reports suggested that physical
trauma may lead to worsening, but in a detailed controlled study no significant asso-
ciations were found between any form of trauma and an increased frequency of
attacks (200). Similarly, surgery and anesthesia do not appear to aggravate the
condition, despite earlier suggestions to the contrary (199,201). A recent review by
a committee of the American Academy of Neurology concluded that physical
trauma was not associated with MS exacerbation (202). However, the group found
that evidence about the relationship to psychological stress was inconclusive. In a
recent meta-analysis of 14 studies, Mohr et al. (203) did find a significant increase
in the risk of exacerbations after stressful life events. However, they were not able
to identify specific stressors.

Measurement of psychological stress and its role in worsening of MS has been
extremely difficult. Methodological problems have included retrospective study
design, which is subject to recall bias; small or highly selected samples; and inade-
quate psychological tests (204–207). Thus, this issue remains unresolved despite
recent studies suggesting that psychological stress may influence the course of MS
(203,207). This could result from changes in the immune system associated with
psychological factors. Interestingly, however, attack rate declined among Israeli
MS patients during the period of SCUD missile attacks on the country in the
Gulf War (208).

Sudden and transient neurological deterioration often results from situations
that elevate body temperature (209). Such worsening is most often associated with
febrile illness, but may also occur with physical exertion. Even moderate exercise
may be associated with an aggravation or precipitation of neurological symptoms,
with blurring of vision most frequently reported (Uhthoff’s phenomenon)
(210,211). Provocation of neurological signs by raised body temperature has been
the basis of a clinical tool, the “hot-bath test” (212). Although seldom performed
today, this technique can be used to uncover additional anatomical lesions in mono-
symptomatic patients. However, a reported patient developed a persistent neurolo-
gical deficit following the test (213). The basis for the neurological worsening
seems to be the development of conduction block in partially demyelinated axons
with elevated temperature (214).

PREGNANCY

For many years, pregnancy was considered to have an adverse effect on the course of
MS. Several modern studies have re-examined this question both retrospectively
(175,215–219) and prospectively (220,221). Pregnancy itself appears to be associated
with a lower exacerbation rate than that in age-matched controls. However, the
postpartum period, particularly the first three months after delivery, is accompanied
by an increase in the frequency of attacks, according to most, but not all, reports
(175,215,217,219–221). In the largest study, Confavreux et al. (222) followed 254
women with MS through 269 pregnancies in 12 European countries. Relapse rates
were lower during pregnancy, most strikingly in the third trimester, when the attack
rate dropped to 0.2 ± 1 compared with 0.7 ± 0.9 during the year before pregnancy
\( P < 0.001 \). However, during the first three months postpartum the rate increased
to 1.2 ± 2.0, significantly greater than that during the year before pregnancy
\( P < 0.001 \). The rate then returned to baseline. Breast-feeding did not have an
adverse effect on relapse rate. These data are consistent with most, but not all, ear-
lier reports. Whether the increased postpartum frequency is related to change in
immunological status after pregnancy, hormonal alteration, the stress of caring for a newborn, or other factors is unknown (219,222).

No current evidence suggests a long-term negative influence of pregnancy on the disease. In fact, recent reports suggest that it may convey a better prognosis (223,224).

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INTRODUCTION

Multiple sclerosis (MS) is the most common chronic inflammatory-demyelinating disease affecting the central nervous system (CNS) of young adults in the western countries, leading, in the majority of cases, to severe and irreversible clinical disability (1). Since its clinical introduction, conventional magnetic resonance imaging (cMRI—dual-echo and postcontrast T1-weighted scans) has greatly improved our ability to diagnose MS and to monitor its evolution, either natural or modified by treatment (Fig. 1) (2). cMRI-derived measures have indeed shown several advantages over clinical assessment, including their more objective nature and increased sensitivity to MS-related changes. Nevertheless, the magnitude of the relationship between cMRI measures of disease activity or burden and the clinical manifestations of the disease is weak (3,4). This necessarily limits the role of cMRI for the understanding of MS pathophysiology and monitoring of experimental treatment.

Several factors are likely to be responsible for this clinical/MRI discrepancy. First, dual-echo imaging lacks specificity with regard to the heterogeneous pathological substrates of individual lesions (3), and, as a consequence, does not allow an accurate quantification of tissue damage. Specifically, edema, inflammation, demyelination, remyelination, gliosis, and axonal loss (5), all lead to a similar appearance of hyperintensity on dual-echo images. This is a major issue now that there is compelling evidence that: (i) inflammatory-demyelination is not enough to explain “fixed” neurological deficits in MS (6); (ii) irreversible axonal damage does occur in inflammed MS lesions (7,8); and (iii) irreversible axonal loss is the main contributor to the clinical manifestations of the disease and to its clinical worsening over time (4,6). Second, T2-weighted images do not delineate tissue damage occurring in the normal-appearing white matter (NAWM), which usually represents a large portion of the brain tissue from MS patients and which is known to be damaged in these patients (9). Postmortem studies have shown subtle changes in the NAWM from
MS patients, which not only include diffuse astrocytic hyperplasia, patchy edema, and perivascular cellular infiltration, but also axonal damage (10–12). Finally, dual-echo imaging does not provide an accurate picture of gray matter (GM) damage, which several pathological studies have shown to be prominent in MS patients.
(13–15) and which is likely to be associated to some clinical manifestations of the disease, such as cognitive impairment and fatigue.

These limitations of dual-echo imaging are only partially overcome by the use of postcontrast T1-weighted scans. Gadolinium (Gd)-enhanced T1-weighted images allow one to distinguish active from inactive lesions (Fig. 1) (16,17), because enhancement occurs as a result of increased blood-brain barrier (BBB) permeability (18) and corresponds to areas with ongoing inflammation (19). However, the activity of the lesions as demonstrated on postcontrast T1-weighted imaging still does not provide information on tissue damage. Chronically hypointense areas on T1-weighted images (Fig. 2) correspond to areas where severe tissue disruption has occurred (20), and their extent is correlated with the clinical severity of the disease and its evolution over time (21,22). Still, measuring the extent of T1 hypointense lesions may not correspond to the severity of intrinsic lesion pathology and provides no information about NAWM and GM damage. Recently, several nonconventional MRI techniques have been developed and applied in an attempt to improve our understanding of the evolution of MS (23). These techniques, including magnetization transfer (MT) MRI, diffusion-weighted (DW) MRI, and proton MR spectroscopy (1H-MRS), can provide quantitative information of MS micro- and macroscopic lesion burden with a higher pathological specificity to the most destructive aspects of MS (i.e., severe demyelination and axonal loss) than cMRI. In addition, their application in longitudinal studies may improve our ability to monitor reparative mechanisms, such as resolution of edema, remyelination, reactive gliosis,

![Figure 2](image.png)

**Figure 2** Axial proton density-weighted (A) and T1-weighted (B) magnetic resonance images of the brain from a patient with a secondary progressive form of multiple sclerosis. In (A), multiple hyperintense lesions are visible with a predominant involvement of the periventricular regions. In (B), some of these lesions are hypointense (“black holes”), indicating that marked tissue destruction (demyelination and axonal loss) has occurred.
and recovery from sublethal axonal injury. Finally, functional MRI (fMRI) holds substantial promise to define the role of adaptive cortical reorganization with the potential to limit the clinical consequences of irreversible MS tissue damage. The present chapter outlines the major contributions obtained by the application of cMRI and modern, quantitative MR-based techniques to the diagnosis of MS and to the understanding of the factors leading to the accumulation of irreversible disability. The main results obtained from the application of MR technology to monitor MS clinical trials are also discussed. These paragraphs are preceded by a brief review of the basic aspects of nonconventional MRI techniques to provide an adequate background to those readers who are not MRI specialists.

A BRIEF REVIEW OF BASIC ASPECTS OF NONCONVENTIONAL MRI TECHNIQUES

MT MRI is based on the interactions between protons in a relatively free environment and those where motion is restricted. Off-resonance irradiation is applied, which saturates the magnetization of the less mobile protons, but this is transferred to the mobile protons, thus reducing the signal intensity from the observable magnetization. Thus, a low MT ratio (MTR) indicates a reduced capacity of the macromolecules in the CNS to exchange magnetization with the surrounding water molecules, reflecting damage to myelin or to the axonal membrane (Fig. 3). The most compelling evidence indicating that markedly decreased MTR values correspond to areas where severe and irreversible tissue loss has occurred comes from a postmortem

![Figure 3](image-url)  
**Figure 3** Axial magnetic resonance images from a patient with multiple sclerosis. The proton density weighted scan (A) shows multiple lesions. On the scalp-stripped magnetization transfer ratio map (B), lesions appear as hypointense areas. The degree of hypointensity is related to decrease in magnetization transfer ratio and indicates damage to myelin and to axonal membranes.
study showing a strong correlation of MTR values from MS lesions and NAWM with the percentage of residual axons and the degree of demyelination (24). Another postmortem study has also shown the potential of this technique to monitor the extent of remyelination in MS lesions (25).

Diffusion is the microscopic random translational motion of molecules in a fluid system. In the CNS, diffusion is influenced by the microstructural components of tissue, including cell membranes and organelles. The diffusion coefficient of biological tissues (which can be measured in vivo by MRI) is, therefore, lower than the diffusion coefficient in free water and for this reason it is named as apparent diffusion coefficient (ADC) (26). Pathological processes that modify tissue integrity, thus resulting in a loss or increased permeability of “restricting” barriers, can determine an increase of the ADC. Since some cellular structures are aligned on the scale of an image pixel, the measurement of diffusion is also dependent on the direction in which diffusion is measured. As a consequence, diffusion measurements can give information about the size, shape, integrity, and orientation of the tissues (27). A measure of diffusion, which is independent of the orientation of structures, is provided by the mean diffusivity (MD), the average of the ADCs measured in three orthogonal directions. A full characterization of diffusion can be obtained in terms of a tensor (28), a $3 \times 3$ matrix which accounts for the correlation existing between molecular displacement along orthogonal directions. From the tensor, it is possible to derive MD, equal to the one-third of its trace, and some other dimensionless indices of anisotropy. One of the most used of these indices is named fractional anisotropy (FA) (29). The pathological elements of MS have the potential to alter the permeability or geometry of structural barriers to water molecular diffusion in the brain (Fig. 4). The application of DW MRI technology to MS is therefore appealing to provide quantitative estimates of the degree of tissue damage and, as a consequence, to improve the understanding of the mechanisms leading to irreversible disability.

Water-suppressed proton MR spectra of normal human brain at long echo times reveal four major resonances: one at 3.2 ppm from tetramethylamines [mainly from choline-containing phospholipids (Cho)], one at 3.0 ppm from creatine (Cr) and phosphocreatine, one at 2.0 ppm from $N$-acetyl groups (mainly NAA), and one at 1.3 ppm from the methyl resonance of lactate (Lac). NAA is a marker of axonal integrity, while Cho and Lac are considered as chemical correlates of acute inflammatory/demyelinating changes (30). An immunopathologic study of MS (31) has indeed shown that a decrease in NAA levels is correlated with axonal loss, and an increase in Cho correlates with the presence of active demyelination and gliosis. $^1$H-MRS studies with shorter echo times can detect additional metabolites, such as lipids and myoinositol (mI), which are also regarded as markers of ongoing myelin damage. Therefore, $^1$H-MRS can complement conventional MRI in the assessment of MS patients by defining simultaneously several chemical correlates of the pathological changes occurring within and outside T2-visible lesions (Fig. 5).

fMRI aids in the mapping of regions of brain activation during motor, sensitive, and cognitive tasks and can define changes in brain activation associated with disease. fMRI quantitates the blood oxygenation level dependent (BOLD) effect and detects areas of brain that have greater local blood flow, reflecting increased neuronal activity during task performance compared with rest (32). As a consequence, fMRI work has the potential to detect adaptive cortical reorganization with the potential to limit the clinical consequences of irreversible MS-related tissue injury.
Figure 4  Axial magnetic resonance images from a patient with multiple sclerosis. (A) Proton density-weighted image. On the scalp-stripped mean diffusivity map (B), some of the lesions appear as hyperintense areas. The degree of hyperintensity is related to mean diffusivity increase and indicates a loss of structural barriers to water molecular motion. On the fractional anisotropy map (C), white matter pixels are bright because of the directionality of the white matter fiber tracts. Dark areas corresponding to some of the macroscopic lesions indicate a loss of fractional anisotropy and suggest the presence of structural disorganization.
The diagnosis of MS is based on the demonstration of disease dissemination in space and time, which can be obtained on a clinical ground (i.e., two relapses in at least two different sites of the CNS) or, alternatively, on the combination of data obtained from clinical assessment and paraclinical and laboratory tests [including MRI, cerebrospinal fluid (CSF) analysis, and evoked potentials (EP)] (33,34). Due to its exquisite sensitivity for the detection of MS lesions at any stage of their evolution, cMRI is very useful for detecting subclinical involvement of multiple CNS sites (i.e., dissemination in space), as well as for detecting clinically silent, newly formed lesions (i.e., dissemination in time), when serial MRI scans are obtained (34). Another major criterion in the diagnostic work-up of patients suspected of having MS is the exclusion of alternative diagnosis. Although a comprehensive review of differential diagnosis of MS is beyond the scope of this chapter and, therefore, readers are referred to standard texts of clinical neurology for a detailed consideration, it is worth mentioning that cMRI also plays a central role in this context. All of this allows a diagnosis of MS to be made earlier and on a more solid ground than with clinical assessment alone.

Typically, all MS lesions appear as areas of increased signal on dual-echo and fast-fluid-attenuated inversion recovery (FLAIR) scans (Fig. 6). On T1-weighed scans, some of these lesions may enhance after Gd injection (Fig. 1) either diffusely (usually newly formed lesions) or ring-like (usually reactivated chronic lesions) (Fig. 7), whereas...
Figure 6  Axial fast-fluid-attenuated inversion recovery images (A and B) from a patient with multiple sclerosis. In (A) and (B), multiple sclerosis lesions appear as areas of increased signal. The suppression of the signal of the cerebrospinal fluid allows a better identification of the lesions located in the periventricular and juxtacortical regions.

Figure 7  Axial proton density-weighted (A) and postcontrast (Gd DTPA, 0.1 mmol/kg) T1-weighted (B) magnetic resonance images of the brain from a patient with multiple sclerosis. In (B), two patterns of enhancement are visible: a homogeneous one (indicating newly formed lesions) (continuous arrow) and a ring-like one (indicating reactivated chronic lesions) (dotted arrow). Abbreviation: Gd, gadolinium.
others may appear as hypointense areas (usually chronic “bum-out” lesions) (Fig. 2).
International consensus has been reached on criteria useful to identify T2 hyperintense (35) and enhancing (36) lesions (Table 1).

MS lesions on MRI scans of the brain are frequently located in the periventricular regions, corpus callosum, and infratentorial areas (with the pons and cerebellum more frequently affected than the medulla and midbrain) (37), and are characterized by oval or elliptical shapes, an uneven distribution across the two hemispheres (contrary to what typically happens in hereditary and metabolic white matter disorders) and variable evolution on serial MRI scans (Fig. 8 and Table 2). In patients with primary progressive (PP) MS, brain lesions are generally few and small in size (38,39).

As shown by several postmortem studies (40–43), the spinal cord is another CNS site frequently involved by MS lesions. Such lesions can be detected by MRI in up to about 90% of patients with established disease (44–49), especially when fast short-tau inversion recovery (fast-STIR) sequences are used (Fig. 9) (49). On the contrary, fast-FLAIR imaging of the cord is only suboptimal (50,51). MRI-detectable lesions are more frequently located in the cervical and thoracic portions of the cord. Several studies identified typical imaging features of MS lesions in the cord (45,49) (Table 2 and Fig. 9): lesions are usually located peripherally, rarely exceed two vertebral segments in length, and occupy less than half of the cord cross-sectional area. Acute lesions are often associated to cord swelling (52), whereas chronic lesions are not hypointense on T1-weighted images, probably because of compact cord tissue organization (53). Enhancing lesions are less frequently seen in the spinal cord than in the brain (54,55), but they are often associated with new clinical symptoms (54). The use of a triple dose (TD) of Gd and delayed postinjection imaging allow for the detection of an increased number of cord lesions (45,56). Asymptomatic cord lesions can be detected in 30% to 40% of patients at presentation with clinically isolated syndromes (CIS) suggestive of MS (57). In relapsing–remitting (RR) MS patients, multiple focal lesions are usually detected (Fig. 9) (45,54,58), whereas in patients with PPMS and secondary progressive (SP) MS, cord abnormalities tend to be confluent (45,58). In addition, PPMS patients may have diffuse hyperintensity throughout the cord (Fig. 9) (48,58). Imaging the spinal cord can be particularly helpful in making a diagnosis of MS in the rare cases where brain MRI is normal or equivocal (59) and in patients more than 50 years old or with nonspecific T2 abnormalities of the brain, because, contrary to what happens for the brain, cord lesions rarely tend to develop with ageing per se (44).

The optic nerve is also frequently involved in the course of MS (Fig. 10). When an optic neuritis (ON) is suspected to be the onset manifestation of MS, the principal role of MRI is assessing the brain for asymptomatic lesions (60–63), whereas optic

<table>
<thead>
<tr>
<th>Table 1</th>
<th>International Consensus Criteria to Identify T2 Hyperintense and Gd-Enhancing Lesions in Multiple Sclerosis</th>
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</thead>
<tbody>
<tr>
<td><strong>T2 lesions, Filippi et al. (35)</strong></td>
<td><strong>Gd-enhancing lesions, Barkhof et al. (36)</strong></td>
</tr>
<tr>
<td>Size &gt; 5 mm</td>
<td>Clear signal contrast vs. NAWM</td>
</tr>
<tr>
<td>Visible on both echoes</td>
<td>Concomitant alteration on T2</td>
</tr>
<tr>
<td>Exclusion of normal anatomical structures</td>
<td>Exclusion of normal anatomical structures</td>
</tr>
<tr>
<td>Exclusion of artifacts and partial volume effects</td>
<td>Exclusion of artifacts and partial volume effects</td>
</tr>
</tbody>
</table>

*Abbreviations: Gd, gadolinium; NAWM, normal-appearing white matter.*
nerve MRI can be useful in ruling out alternative diagnoses. The sensitivity of MRI for detecting optic nerve lesions in patients with ON is high: a seminal study using a STIR sequence showed lesions in 84% of symptomatic nerves and 20% of asymptomatic nerves (64). Recently, the use of fat-saturated fast spin echo (65) and selective partial inversion recovery prepulse (SPIR)-FLAIR (66) sequences have led to increases in sensitivity for detecting lesions in patients with an ON. In detail, SPIR-FLAIR sequences have shown an increased detection rate of lesions in the optic nerve and peripapillary retina. These findings highlight the importance of imaging techniques in the diagnosis and management of ON. Figure 8 shows axial proton density-weighted images from a patient with multiple sclerosis (A–D). In all the sections, multiple hyperintense lesions are visible. These lesions are located in the infratentorial areas (A), the periventricular regions (B and C), the corpus callosum (B and C), and juxtacortical areas (D), and some of them have an oval or an elliptical shape. They are also unevenly distributed across the two hemispheres.
Table 2  Main Magnetic Resonance Imaging Features of Brain and Cord Lesions of Multiple Sclerosis

<table>
<thead>
<tr>
<th>Brain lesions</th>
<th>Spinal cord lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location: periventricular, infratentorial, corpus</td>
<td>Best seen on fast-STIR scans</td>
</tr>
<tr>
<td>callosum, juxtacortical</td>
<td>Mostly located in the cervical cord</td>
</tr>
<tr>
<td>Shape: irregular, ovoid</td>
<td>Small (&gt;1–2 vertebral segments)</td>
</tr>
<tr>
<td>Distribution: asymmetric</td>
<td>Do not occupy the entire cord cross-sectional area</td>
</tr>
<tr>
<td>Evolution: variable</td>
<td>Rarely visible as black holes</td>
</tr>
</tbody>
</table>

Abbreviation: STIR, short-tau-inversion recovery.

Figure 9  Sagittal fast short-tau-inversion recovery images of the cervical cord from a patient with relapsing–remitting (A) and a patient with primary progressive (B) multiple sclerosis. In (A), two hyperintense lesions, one at C3 to C4 and one at C7 to C8, are visible. In (B), a diffuse hyperintensity extending from C2 to C4 can be seen.
and SPIR-FLAIR allowed to detect lesions in all symptomatic nerves imaged, and lesion lengths were also largest on SPIR-FLAIR images (66). In MS patients, increased T2 signal can be seen for a long time after an episode of ON, despite improvements in vision and visual EP, and even in the absence of acute attacks of ON (67). T1 hypointense lesions are not seen in the optic nerve (53), whereas Gd

Figure 10 Enhanced fat-saturated T1-weighted image (A–C) showing enhancement in the left optic nerve (arrow) due to acute optic neuritis in a patient with multiple sclerosis.
enhancement is a consistent feature of acute ON (68,69). In MS, the use of a TD of Gd improves lesion detection in the optic nerve (53).

On the basis of these observations, in the past two decades, a number of MRI criteria have been proposed (2,70,71) to increase the confidence in making a diagnosis of MS:

- Criteria of Paty et al. (70): presence of at least four T2-hyperintense lesions or three T2 lesions, of which one is periventricular. These criteria are characterized by high sensitivity but relatively low specificity (72).
- Criteria of Fazekas et al. (2): presence of at least three T2-hyperintense lesions with two of the following characteristics: an infratentorial lesion, a periventricular lesion, and a lesion larger than 6 mm. These criteria showed both high sensitivity and high specificity when evaluated retrospectively in definite MS (73), but perform less well in a prospective fashion when applied in CIS patients (74).
- Criteria of Barkhof et al. (71): presence of at least three of the four following features: presence of at least one Gd-enhancing lesion, at least one juxtacortical lesion, at least one infratentorial lesion and three or more periventricular lesions. In 2000, Tintore et al. (75) slightly modified these criteria by allowing for nine T2 lesions to be an alternative for the presence of an enhancing lesion and reported a high specificity of these criteria for CIS patients converting to clinically definite (CD) MS.

The most recent diagnostic criteria for MS (34), proposed by an International Panel (IP) of MS specialists, rely on an objective evidence of lesion dissemination in space and time, as did the previous ones by Poser et al. (33). As a consequence, cMRI of the brain gained an additional role in the diagnostic work-up of patients suspected of having MS. For the demonstration of dissemination in space, the IP decided to apply the modified Barkhof-Tintore criteria. When these more stringent imaging criteria are not fulfilled, the IP allowed the presence of at least two T2 lesions plus the presence of oligoclonal bands in the CSF. However, Tintore et al. (76) recently suggested that this alternative criterion may result in a decreased diagnostic accuracy, since they reported in CIS patients followed for three years a specificity of only 63% for the development of CDMS. In the IP criteria (34), temporal dissemination of the disease can be demonstrated either by the presence of at least one enhancing lesion on an MRI scan performed three months or more after the onset of the clinical event or by the presence of a new T2 or an enhancing lesion on an MRI scan performed six months or more after the onset of the clinical event (Fig. 11).

The major advantage of the IP criteria (34) is that they allow to make an early diagnosis of MS in patients with a clinically isolated attack. In a three year follow-up study of CIS patients, Dalton et al. (77) tested the ability of the new criteria to predict conversion to CDMS and found a sensitivity, specificity and accuracy of 83%. These results were confirmed by Tintore et al. (76), who reported a sensitivity of 74%, specificity of 86%, and accuracy of 80%. In the placebo arm of a trial of patients at the earliest clinical stage of MS, the IP criteria for dissemination in space also worked quite well in predicting subsequent evolution to CDMS (78). However, it is worth noting that the MRI spatial dissemination criteria are not as specific in predicting conversion to CDMS in patients presenting with a CIS of the brain stem (79). The presence of asymptomatic cord lesions was found to help in the demonstration of spatial dissemination in recently diagnosed MS patients (80), but the substitution of a brain lesion with a cord lesion did not impact significantly on subsequent diagnosis in patients presenting with ON (81). When a new T2 lesion was allowed as
evidence for dissemination in time, one study showed that 82% of CIS patients who fulfilled the new MRI criteria for MS after three months had developed CDMS after three years (82), and another found that 80% of those CIS who fulfilled the same criteria after one year developed CDMS after three years (76).

Figure 11 Axial proton density–weighted images of the brain from a patient at presentation with a clinically isolated syndrome suggestive of multiple sclerosis, at baseline (A) and after a follow-up of six months (B). In (B), a new hyperintense periventricular lesion is visible. On the postcontrast T1-weighted scan obtained after six months from disease onset (C), this lesion is enhanced. This demonstrates disease dissemination in time.
Due to the relatively recent introduction of MRI in the clinical assessment of patients with MS, there are only a few mid- and long-term studies that investigated the prognostic role of this technique in CIS patients (62,63,83). Although the results of such studies are likely to be affected by dropouts and relatively poor image resolution at study entry, it can be stated that the presence of one asymptomatic T2 lesion on MRI of the brain at presentation is associated with an increased likelihood that MS will develop in the subsequent 5 to 14 years (62,63,83,84). The study with the longest follow-up (63) showed that, after a mean of 14.1 years, CDMS developed in 88% of CIS patients with abnormal MRI at presentation and in only 19% of those with normal MRI scans. These data have been recently confirmed by Minneboo et al. (83), who showed, after a median follow-up of 8.7 years, conversion to CDMS in 62% of CIS patients with abnormal MRI at presentation. These results, on the one hand, indicate that demyelinating events may remain monophasic even in patients with MRI evidence of disease dissemination in space and after moderately long-term follow-up and, on the other, that a normal MRI scan at presentation does not rule out MS completely in CIS patients.

Contrary to what happens for subsequent clinical relapses, the number (volume) of T2 lesions on baseline MRI scans from a CIS patient increases the likelihood of developing “fixed” disability on the subsequent 5 to 14 years (63,83). In the study by Brex et al. (63), analysis of lesion loads at years 5, 10, and 14 also showed that the strongest correlation of final disability was with the year 5 measure, and that subsequent changes in lesion load appeared less relevant. In the study by Minneboo et al. (83), EDSS 3 was reached in 48% of patients with four or more lesions at presentation, and in 55% of patients with 10 or more lesions. Minneboo et al. (83) also showed that the likelihood to reach EDSS 3 was best predicted by the presence of at least two infratentorial lesions, suggesting that the distribution of lesions may also be an important prognostic factor.

Recent MRI studies have shown that irreversible tissue loss/damage is an early event in the course of MS (85–101). Although definitive data are still lacking, it is likely that the extent of such irreversible tissue damage might also convey important prognostic information. Dalton et al. (85) prospectively followed 55 CIS patients for three years: after the first year (86), patients who evolved to MS according to the IP criteria (34) developed significantly more ventricular enlargement than did those without disease evolution; after three years (85), 53% of the patients had evolved to MS, and at this time, increased ventricular volume and GM atrophy were found in patients developing MS compared to those who did not evolve. Similar findings were also demonstrated when brain atrophy was measured in a trial of patients at the earliest clinical stage of MS (87): mean percentage brain volume changes for patients on placebo was \(-0.83\)% during the first year, \(-0.67\)% during the second year, and \(-1.68\)% during the entire study period; corresponding values for treated patients were \(-0.62\)% , \(-0.61\)% , and \(-1.18\)% , respectively. The changes in brain volume were significant in both groups at all timepoints. Compared to normal controls, cord area was found to be only slightly reduced in patients at presentation with CIS and an abnormal MRI scan, but cord area remained stable over one year after disease onset (88). MRI-detectable atrophy of the optic nerves is also seen following an episode of ON (89–92).

Reduced MTR values have been detected in the normal-appearing brain tissue (NABT) from patients at presentation with CIS (93,94), and the extent of these abnormalities was reported to be an independent predictor of subsequent disease evolution in one of these studies (93). However, these observations were not confirmed by later studies (95,96). Recent works in CIS patients have found no
abnormality in cervical cord MTR (97) but abnormal measures of DW in NAWM (98), which however were not predictive of subsequent lesion dissemination in time (as defined by McDonald criteria) at 3 and 12 months (98). These MTR and diffusion findings suggest that subtle NAWM brain damage may occur at a very early stage in CIS patients, but may not predict short-term lesion development.

Two recent 1H-MRS studies (99,100) have shown that metabolic changes may also occur in patients at the earliest clinical stage of MS. Filippi et al. (99) demonstrated a reduction in the concentration of NAA of the whole brain, whereas Fernando et al. (100) found increased mI and Cr in NAWM. These studies suggest that axonal pathology and glial proliferation can be early events in MS. In CIS patients, nonconventional MRI quantities might reflect clinical status better than does lesion load. Recently, Arevalo et al. (101) reported a decrease of NAA/Cr ratio and parenchymal fraction of brain in cognitively impaired CIS patients when compared with CIS patients with normal cognitive performance, whereas no difference was found between the two groups in terms of cMRI metrics.

Using fMRI, an abnormal pattern of movement-associated cortical activation has also been described in CIS patients within three months from disease onset (102,103). In a one year follow-up study of CIS patients (104), those who developed CDMS had a different motor fMRI response at first presentation when compared with those who did not (Fig. 12), suggesting that activation of the regions classically involved in the performance of a given task seems to be a favorable prognostic factor, whereas a widespread recruitment of additional areas seems to be associated with short-term disease evolution.

THE ROLE OF MRI IN UNDERSTANDING MS PATHOPHYSIOLOGY

cMRI is not only important for diagnosing MS but also in giving clues about MS pathophysiology, as outlined by the following findings:

1. The patterns of MRI activity vary significantly in individual patients over time, from one patient to another, and across the different clinical phenotypes of MS. Disease activity tends to decline with patients’ age (105) and is very low in patients with PPMS (106,107).
2. The harvest of enhancing MS lesions can be markedly increased when administering a TD of Gd (108). Since those lesions enhancing only after a TD are likely to represent areas with mildly increased BBB permeability (109), the simultaneous presence of lesions enhancing at different Gd doses suggests that the severity of MRI-detectable inflammation is highly variable among lesions from the same MS patients.
3. Patients with SPMS usually have high T1 hypointense lesion load (22,110). In these patients, the volume of “black holes” correlates better than the T2 lesion load with disability (20,22).
4. Significant reductions of brain volume and cervical cord size can be observed even in the early phase of MS (87,88,111,112). The severity of brain and cord atrophy is, however, more pronounced in the progressive forms of MS (Fig. 13) (44,47,111,113,114) and can worsen in the absence of MRI-visible disease activity (110,112,115). Cord cross-sectional area and its change over time correlate better with clinical disability than T2-visible burden (113,116).
Progressive cord and brain atrophy have been observed over a five-year period in PPMS (117), but the lack of correlation between the two suggests that independent processes may be contributing to progressive tissue loss in the two regions. Significant GM atrophy has also been detected in patients with MS (85,111,118) and has been found to correlate with the severity of cognitive impairment (119).

Atrophy of the optic nerve following an episode of ON can be detected using MRI techniques.

**Figure 12** Relative cortical activations on a rendered brain during the performance of a simple motor task with the dominant, functionally unaffected right hand in patients with clinically isolated syndromes suggestive of multiple sclerosis who evolved to definite multiple sclerosis over a short-term follow-up period (A and B) compared with those who did not (C and D). When compared with (C and D), in (A and B), a more extensive and widespread activation of the sensorimotor network is visible.
cMRI (89–92). After an initial swelling during the acute phase of ON, the mean area of diseased optic nerves significantly decreases over a one-year follow-up (90,92).

Due to the increased pathological specificity to the most destructive aspects of MS pathology and to the ability to quantify subtle tissue damage in the normal-appearing tissue (4), modern quantitative MR techniques are increasing dramatically our understanding of MS pathophysiology, which complements the information derived from the application of cMRI, as outlined in the following paragraphs.

**Quantification of Intrinsic Lesion Damage**

In chronic T2-hyperintense lesions, MT MRI and DW MRI studies have shown variable degrees of MTR, FA and NAA reduction and MD increase (4,120,121). All these values vary dramatically across individual lesions. These abnormalities are more pronounced in lesions that are hypointense on T1-weighted images and in patients with the most disabling courses of the disease (4,120,122,123). The variability of MTR, MD, and FA values seen in MS lesions suggests that different proportions of lesions, with different degrees of structural damage might contribute to the evolution of the disease. This concept is supported by a three-year follow-up study (124) showing that newly formed lesions from SPMS patients have more severe MTR deterioration than those from mildly disabling RRMS patients.

New enhancing lesions have different range of MTR values, according to their size, modality, and duration of enhancement. In particular, MTR is higher in homogeneously enhancing lesions than in ring-enhancing lesions (125); in lesions enhancing on a single scan than in those enhancing on two or more serial scans (108) and in lesions enhancing after the injection of a TD of Gd than in those enhancing after the injection of a standard dose (109). DW MRI characteristics of enhancing lesions are less well defined. While FA values are consistently lower in enhancing

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**Figure 13** Axial T1-weighted magnetic resonance images of the brain from a normal control patient (A) and from a patient with secondary progressive multiple sclerosis (B). In the multiple sclerosis patient, an enlargement of the lateral ventricles and of the brain sulci is evident.
than in nonenhancing lesions (126,127), conflicting results have been achieved when comparing ADC or MD between these two lesion populations. While some studies reported higher ADC or MD values in nonenhancing than in enhancing lesions (126,128), others, based on larger samples of patients and lesions, did not report any significant difference between the two lesion populations (127,129). The heterogeneity of enhancing lesions has been also underlined by the demonstration that water diffusivity is markedly increased in ring-enhancing lesions when compared with homogeneously enhancing lesions (130), or in the nonenhancing portions of enhancing lesions when compared with the enhancing portions (130).

\(^1\)H-MRS of acute MS lesions at both short and long echo times reveals increases in Cho and Lac resonance intensities (121,131), which reflect the releasing of membrane phospholipids and the metabolism of inflammatory cells, respectively. In large acute demyelinating lesion decreases of Cr can also be seen (121). Short echo time spectra can detect transient increases in visible lipids released during myelin breakdown and mI (132). All these changes are usually associated with a decrease in NAA. After the acute phase and over a period of days to weeks there is a progressive reduction of raised Lac resonance intensities to normal levels. Resonance intensities of Cr also return to normal within a few days. Cho, lipid, and mI resonance intensities return to normal over months. The signal intensity of NAA may remain decreased or show partial recovery, starting soon after the acute phase and lasting for several months (121,131,133).

A progressive decrease of MTR values and an increase of MD values can be detected in regions that will develop new lesions (134–139). Using \(^1\)H-MRS, Cho increase, probably reflecting an altered myelin chemistry or the presence of inflammation, and a decrease in NAA have been also shown in prelesional NAWM (132,140,141).

Average lesion MTR has been found to be lower in patients with RRMS than in those with CIS suggestive of MS (93,142), whereas no differences have been found in cross-sectional studies between patients with RRMS and those with SPMS (142) or between patients with SPMS and those with PPMS (110).

**Assessment of NABT and NAWM Damage**

The quantification of the extent of NAWM and NABT involvement in MS can be obtained using either a region-of-interest (ROI) analysis or an histogram-based approach. While the main advantage of ROI analysis is that it enables to obtain detailed information on the characteristics of clinically eloquent NAWM sites, using histogram analysis, the amount of operator intervention is reduced, thus limiting both the measurement variability in serial studies and the time needed for the analysis. In addition, the recent development of fully automated techniques to segment the various components of brain parenchyma has enabled us to obtain histograms of the NAWM in isolation, by preliminarily excluding from the analysis those pixels belonging to T2-visible lesions and GM.

Using ROI analysis, reduced MTR, FA, and NAA and increased ADC and MD values have been shown in the NAWM of MS patients with all the major MS phenotypes (99,126–129,138–140,143–150). Diffusely elevated Cho, Cr, and Ins concentrations have been described in the NAWM of RRMS (151,152) and PPMS (153) patients. Elevated levels of Ins have also been detected in the NAWM of patients with early RRMS (112) and in patients at presentation with CIS suggestive of MS (100). MTR changes, of a lower magnitude than those observed in T2-visible lesions, have been detected in the dirty-appearing white matter of MS patients (154).
The application of histogram analysis (93,94,110,142,147,155–157) to the study of the NABT and of the NAWM confirmed and extended previous findings obtained with ROI analysis, showing that these abnormalities can be detected even in patients with CIS suggestive of MS (93,94,98) and in those with early onset MS (158), are more pronounced in SPMS and PPMS patients than in patients with the other disease phenotypes (156), and are similar between patients with SPMS and those with PPMS (110). Consistent with this is the demonstration that NAA reduction is more pronounced in the NAWM of SPMS and PPMS patients than in those with RRMS (149,153). Nevertheless, reduced NAWM NAA can also be detected in patients with no overt clinical disability (150) and in those in the early phase of the disease (159). The recent development of an unlocalized 1H-MRS sequence for measuring NAA concentration in the whole brain (WBNAA) (160) has allowed to extend the previous findings by showing the presence of marked axonal pathology in CDMS (161–164) and in patients at the earliest clinical phase of MS (99).

On average, NABT changes tend to worsen over time in all MS phenotypes (93,149,165–167), including patients with PPMS (168), even if these changes seem to be more pronounced in SPMS patients (165). In patients with established MS, NAWM MTR reduction has been shown to predict the accumulation of clinical disability over the subsequent five years (166,167). In patients with RRMS, longitudinal decrease over time of NAA/Cr in the NAWM correlates strongly with EDSS worsening (149,169), suggesting that progressive axonal damage or loss may be responsible for functional impairment in MS. More recently, it has been demonstrated that brain axonal damage begins early in the course of MS, develops more rapidly in the earlier than in the later clinical stages of the disease and correlates more strongly with disability in patients with mild than in those with more severe disease (159).

NABT MTR, MD, and NAA values are only partially correlated with the extent of macroscopic lesions and the severity of intrinsic lesion damage (93,99,127,129,147,162,170–172), thus suggesting that NABT pathology does not only reflect Wallerian degeneration of axons traversing large focal abnormalities, but they may also represent small focal abnormalities beyond the resolution of conventional scanning and independent of larger lesions.

The quantification of the extent of NABT and NAWM involvement has allowed to increase the strength of the relationship between MRI metrics and the clinical manifestations of the disease. Moderate to strong correlations between various brain MTR and MD histogram-derived metrics and the severity of disability have been shown by several studies (147,155,173–177). These correlations have been found to be stronger in patients with RRMS and SPMS than in other disease phenotypes (155,174). Subtle MTR changes in the NABT (178,179) and in the cortical/subcortical (180) brain tissue are well correlated with the presence of neuropsychological impairment in MS patients. In addition, a multivariate analysis of several cMRI and MT MRI variables has demonstrated that average NABT-MTR is more strongly associated to cognitive impairment in MS patients than the extent of T2-visible lesions and their intrinsic tissue damage (181). The reduction of NAA/Cr ratio in the NAWM of MS patients has been found to correlate with the presence of fatigue (182).

MT MRI, DW MRI, and 1H-MRS metrics of specific brain structures, such as the cerebellum (148,173,183), the brainstem (173) or the pyramidal tracts (182–186) of MS patients are significantly associated with impairment of these functional systems. Recently, Gadea et al. (187) found a relationship between attentional dysfunction in early RRMS patients and NAA/Cr values in the locus coeruleus nuclei of the pontine ascending reticular activation system.
Assessment of GM Damage

Using ROI (172) and histogram analysis (172,188–192), MT MRI and DW MRI abnormalities have been shown in the GM of MS patients, including those with PPMS (188,190), whereas no MD abnormalities have been detected in the GM of patients at presentation with CIS (98). Although GM changes are more pronounced in patients with SPMS than in those with RRMS (190,192), a recent 18-month follow-up study has shown that GM damage increases with time in RRMS patients (Fig. 14) (193). This suggests a progressive accumulation of GM damage already in the RR phase of the disease, which was previously unrecognized and which might be one of the factors responsible for the development of brain atrophy (111). No difference in the extent and severity of GM involvement has been found between patients with SPMS and those with PPMS (188).

Metabolite abnormalities, including decrease of NAA, Cho, and glutamate, have also been shown in the cortical GM of MS patients (152,194–196) since the early phases of the disease (194), but not in CIS patients (197). These changes are more pronounced in patients with SPMS than in those with RRMS (195,198). Reduced

![Figure 14](image-url)

**Figure 14** Average mean diffusivity (A) and mean diffusivity histogram peak height (B) of gray matter during an 18-month follow-up study of patients with relapsing–remitting multiple sclerosis where diffusion-weighted magnetic resonance imaging was obtained at baseline and then every three months. Vertical bars represent 95% confidence intervals. Continuous lines represent the linear trends for each variable, as resulting from the respective time trend analyses. Mean diffusivity is expressed in mm²/sec.
NAA and increased ADC have also been demonstrated in the thalamus of SPMS (199,200) and RRMS (200,201) patients. As shown for cortical changes, deep GM abnormalities are also more pronounced in SPMS than in RRMS patients (200).

Significant correlations have been reported between MT MRI and DW MRI changes and T2-lesion volume (172,188,189,192). This fits with the notion that at least part of the GM pathology in MS is secondary to retrograde degeneration of fibers traversing WM lesions.

A precise and accurate quantification of GM damage might help to explain some of the clinical manifestations of MS, such as cognitive impairment, and might contribute to increase the strength of the correlation between clinical and MRI findings. Recent studies have indeed found a correlation between the severity of cognitive impairment, and the degree of MTR (180) and MD (177) changes in the GM of MS patients. In addition, GM MTR metrics have been shown to correlate with the severity of clinical disability in patients with RRMS (189) and PPMS (191). Disappointingly, no correlation has been demonstrated between the extent of GM pathology, measured using MT MRI and DW MRI, and severity of fatigue (202).

Assessment of Optic Nerve and Spinal Cord Damage

In addition to atrophy measurements, reliable MTR measurements can be obtained from the optic nerve and spinal cord, which shows the feasibility of the application of MT MRI for the assessment of the involvement of these critical structures in the course of MS.

Two ROI-based studies (203,204) reported abnormal MTR values in the optic nerve after an acute ON, independently of the presence of T2-visible abnormalities (204). Inglese et al. (91) demonstrated a correlation between MTR changes and the degree of visual function recovery after an acute episode of ON in 30 MS patients, showing that MTR reduction was more pronounced in the optic nerves of MS patients with no recovery than in those with clinical recovery, and that similar reductions were seen in patients with Leber’s hereditary optic neuropathy, indicating that axonal loss is likely to be an important contributor to MTR decrease in MS. In a serial MTR study of patients with acute ON, Hickman et al. (92) showed that the MTR of the affected optic nerves was not different from that of optic nerves from normal controls during the acute phase, but it declined over time significantly with a nadir at about eight months after disease onset, despite the rapid initial visual recovery. The MTR decline is consistent with demyelination and axonal damage; the late nadir may have been due to slow clearance of myelin debris. Subsequently, diseased optic nerve MTR appeared to rise, possibly due to remyelination. Although more technically demanding, successful DW MRI of the optic nerve (205,206) has also been obtained in healthy individuals (205,206) and MS patients (205). Iwasawa et al. (205) assessed water diffusion in the optic nerves of patients with ON, demonstrating significant different optic nerve ADC values between controls and patients. In addition, this study demonstrated that ADC values are decreased in the acute (inflammatory) stage of ON and increased in the chronic phase.

Using ROI analysis, Silver et al. (207) found reduced MTR values in the cervical cord of 12 MS patients in comparison with healthy volunteers; however, no correlation was found between cord MTR and disability, probably due to the small number of subjects enrolled and the limited portion of cord studied. These results have been partially confirmed by a subsequent study performed on 65 MS patients (208), where a weak correlation between the reduction of MTR values and the...
increase of clinical disability has been found. More recently, the use of histogram analysis has allowed to obtain a more global picture of cord pathology in patients with MS. Histogram analysis has demonstrated that cord MTR histogram metrics in CIS (98), RRMS (209), and early-onset MS (158) patients are similar to those of healthy individuals. On the contrary, cord MTR metrics are markedly reduced in patients with SPMS and PPMS (110,210). Average cervical cord MTR is lower in MS patients with locomotor disability than in those without (209). In PPMS, a model including cord area and cord MTR histogram peak height was significantly, albeit modestly, associated with the degree of disability (110). In patients with MS, cord MTR is only partially correlated with brain MTR (210), suggesting that MS pathology in the cord is not a mere reflection of brain pathology and, as a consequence, measuring cord pathology might be a rewarding exercise in terms of understanding MS pathophysiology.

With increasing technical advances, it has also become possible to study cord MS pathology using DW MRI (211–216). A preliminary study, which assessed water diffusion in seven cord lesions of three MS patients with locomotor disability (213), found increased MD values in MS cord lesions in comparison to the cord tissue from healthy volunteers. More recently, Filippi et al. (215) used histogram analysis to assess water molecular diffusivity of the cervical cord from 44 patients with either RRMS or SPMS and found reduced average cord FA in MS patients compared with controls (Fig. 15). In MS, the reduction of cord FA was correlated with the degree of disability. Altered MD and FA cord histogram derived metrics have also been found in patients with PPMS (216).

**Mechanisms of Recovery**

In MS, several mechanisms have the potential to cause tissue injury and, as a consequence, several mechanisms of recovery can also be advocated. Although our ability to monitor recovery using MR is still limited, it is certain that such a goal would represent a major achievement in our understanding of the disease and the assessment of treatment efficacy. Table 3 summarizes some of the damaging/recovery aspects of MS in relation to MR techniques with the potential to provide estimates of tissue repair (if used in a longitudinal fashion and at appropriate time intervals).

In case of severe and irreversible neuroaxonal damage, cortical reorganization might represent a major contributor in promoting functional recovery. Given the importance of the “axonal hypothesis” in the pathophysiology of MS (6) and the fact that fMRI literature in MS is rapidly increasing, the rest of this paragraph reviews the major results obtained using MR technology in defining the role of cortical reorganization in limiting the functional consequences of MS-related tissue injury.

Functional cortical changes have been demonstrated in all MS phenotypes, using different fMRI paradigms. A study of the visual system (217), in patients who had recovered from a single episode of acute ON, demonstrated that such patients had an extensive activation of the visual network compared with healthy volunteers. An altered brain pattern of movement-associated cortical activations, characterized by an increased recruitment of the contralateral primary sensorimotor cortex (SMC) during the performance of simple tasks (102,103) and by the recruitment of additional “classical” and “higher-order” sensorimotor areas during the performance of more complex tasks (103) has been demonstrated in patients with CIS. An increased recruitment of several sensorimotor areas, mainly located in the cerebral hemisphere ipsilateral to the limb that performed the task has also been shown in patients with early MS and a previous episode of hemiparesis (218). In
patients with similar characteristics, but who presented with an ON, this increased recruitment involved sensorimotor areas which were mainly located in the contralateral cerebral hemisphere (219). In patients with established MS and a RR course, functional cortical changes have been shown during the performance of visual (220), motor (Fig. 16) (221–225), and cognitive (226–229) tasks. Movement-associated cortical changes, characterized by the activation of highly specialized cortical areas, have also been described in patients with SPMS (230) during the performance of a simple motor task. Finally, two fMRI studies of the motor system (225,231) of patients with PPMS suggested a lack of “classical” adaptive mechanisms as a potential additional factor contributing to the accumulation of disability. The results of all these studies suggest that there might be a “natural history” of the functional reorganization of the cerebral cortex in MS patients, which might be characterized, at the beginning of the disease, by an increased recruitment of those areas “normally” devoted to the performance of a given task, such as the primary SMC and the supplementary motor area (SMA) in case of a motor task. At a later stage, bilateral

![Sagittal diffusion tensor magnetic resonance image of the cervical cord from a patient with relapsing–remitting multiple sclerosis](image)

**Figure 15** Sagittal diffusion tensor magnetic resonance image of the cervical cord from a patient with relapsing–remitting multiple sclerosis: mean diffusivity map (A), fractional anisotropy map (B), and color-encoded map of directionality (dark gray color means a preferential fiber direction along the z-axis, gray color along the x-axis, light gray color along the y-axis). The loss of normal fiber directionality is visible (C).
The activation of these regions is first seen, followed by a widespread recruitment of additional areas, which are usually recruited in normal people to perform novel/complex tasks. This notion has been supported by the results of a recent study (232), which has provided a direct demonstration that MS patients, during the performance of a simple motor task, activate cortical regions that are part of a fronto-parietal circuit, the activation of which typically occurs in healthy subjects during object manipulation.

Functional and structural changes of the MS brain are strictly correlated (Fig. 17). Several moderate to strong correlations have been demonstrated between the activity of cortical and subcortical areas and the extent of brain T2-visible lesions (218,221,224,230,231), the severity of intrinsic lesion damage (219,224), the severity of NABT damage, measured using 1H-MRS (102,222), MT MRI or DW MRI (224,225,230), the involvement of specific white matter tracts, such as the pyramidal tract (233), the extent of GM damage (231,234) and, finally, the severity of cervical cord damage (225,235).

Although the actual role of cortical reorganization on the clinical manifestations of MS remains unclear, there are several pieces of evidence, in addition to the strong correlation found between functional and structural abnormalities, that suggest that cortical adaptive changes are likely to contribute in limiting the clinical consequences of MS-related structural damage. In detail, in a patient with an acute hemiparesis following a new, large demyelinating lesion located in the corticospinal tract, dynamic changes of the brain pattern of activation of the “classical” motor areas, ending in a full recovery of function, have been observed (223). The correlation found between the extent of functional cortical changes and NAA levels suggests that dynamic reorganization of the motor cortex can occur in response to axonal injury associated with MS activity. In patients complaining of fatigue, when

### Table 3  Main Damaging/Recovery Aspects of Multiple Sclerosis and Magnetic Resonance Imaging Techniques with the Potential to Provide Estimates of Tissue Repair

<table>
<thead>
<tr>
<th>Tissue injury</th>
<th>Mechanisms of repair</th>
<th>MRI metrics</th>
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<tbody>
<tr>
<td>Acute cytokine release with intact myelination and preserved axons</td>
<td>Removal of inflammatory mediators</td>
<td>Ceasement of Gd enhancement Disappearance of the Lac peak and increase of Cr on 1H-MRS Generalized increase of all metabolites peaks on 1H-MRS Increase of MTR</td>
</tr>
<tr>
<td>Demyelination</td>
<td>Remyelination Redistribution of Na(^+) channels on persistently demyelinated axons</td>
<td>Disappearance/reduction of T1 hypointensities Marked increase of MTR Reduction of Cho, mI, and lipid peaks on 1H-MRS Reduction of MD with normal FA</td>
</tr>
<tr>
<td>Sublethal axonal injury</td>
<td>Recovery of neuro-axonal function</td>
<td>Increase of the NAA peak on 1H-MRS</td>
</tr>
<tr>
<td>Irreversible tissue loss</td>
<td>Reactive gliosis</td>
<td>Decreased FA with normal MD</td>
</tr>
<tr>
<td>Irreversible neuro-axonal damage</td>
<td>Cortical reorganization</td>
<td>Increased and widespread cortical recruitment</td>
</tr>
</tbody>
</table>

**Abbreviations:** Gd, gadolinium; 1H-MRS, proton magnetic resonance spectroscopy; Lac, lactate; MTR, magnetization transfer ratio; DW MRI, diffusion-weighted magnetic resonance imaging; Cho, choline; mI, myoinositol; NAA, N-acetylaspartate; MD, mean diffusivity; FA, fractional anisotropy.
compared with matched nonfatigued MS patients (236), a reduced activation of a complex movement-associated cortical/subcortical network, including the cerebellum, the rolandic operculum, the thalamus, and the middle frontal gyrus has been found. In fatigued patients, a strong correlation between the reduction of thalamic activity and the clinical severity of fatigue was also found, suggesting that a less marked cortical recruitment might be associated to the appearance of clinical symptomatology in MS (Fig. 18). Finally, preliminary work has shown that the pattern of movement-associated cortical activations in MS is determined by both the extent of brain injury and disability, and that these changes are distinct (237).

**MRI IN MONITORING TREATMENT EFFICACY IN MS TRIALS**

cMRI-derived end-points have been used as primary and secondary outcome measures for monitoring MS clinical trials (Table 4) (3,4,238–240). In this context, the
The most widely used cMRI measures are those reflecting disease activity (new or enlarged T2 lesion counts, enhancing and new enhancing lesion counts, enhancing lesion volume measurement) and accumulated disease burden (T2 lesion load assessment). Over the past decade, a large number of parallel group, placebo-controlled and baseline versus treatment trials have unambiguously shown the ability of several immunomodulating and immunosuppressive treatments to reduce both MRI-measured inflammation and the consequent increase of accumulated lesion burden in patients with CIS, RRMS, and SPMS (241).

Some trials have also investigated the effect of treatment in preventing the accumulation of T1 black holes (242–245) or the development of brain atrophy (87,115,246–252). In RRMS and SPMS, these studies have consistently shown that the effect, if any, of all the tested treatments in reducing the rate of accumulation of black holes or the rate of development of brain atrophy was moderate at best, even when the same treatment was highly effective on MRI measures of MS activity (253). The situation seems to be different in patients at the earliest clinical stage of MS, where a low dose of IFN beta-1a given subcutaneously once a week has shown to be able to reduce accumulation of brain atrophy by about 30% in two years (87). Nevertheless, even in such patients, as it is the case for those with RRMS and SPMS, the magnitude of the correlation between MRI-detectable inflammation and neurodegeneration remains poor (253), suggesting a mismatch between the two major pathological aspects of the disease since its onset onwards. Two studies have evaluated the effect of glatiramer acetate (GA) (254) and interferon (IFN) beta-1b (255) on the probability of newly formed MS lesions to evolve into chronically T1 hypointense lesions. Although this approach is highly time consuming, it sounds promising for assessing in a relatively short time the ability of a given treatment to alter

**Figure 17** (A) Scatterplot of the correlation between the relative activation of the contralateral primary sensorimotor cortex and average lesion mean diffusivity in nondisabled relapsing–remitting multiple sclerosis patients during a simple motor task. (B) Scatterplot of the correlation between the relative activation of the contralateral infraparietal sulcus and normal appearing brain tissue average mean diffusivity in nondisabled relapsing–remitting multiple sclerosis patients during a simple motor task.
favorably the mechanisms leading to irreversible tissue loss. New approaches have been suggested to improve the sensitivity of cMRI in detecting disease activity (108) or irreversible tissue loss (3,4,111). TD MRI might be useful to grade the efficacy of experimental treatment on MRI-detectable inflammation (256–258) and to reduce the sample sizes and follow-up periods needed to achieve a given study power (108,259). Although the optimization and standardization across multiple sites and over time of MT sequences might be challenging and long-term longitudinal studies using MT MRI are lacking, MT MRI holds substantial promise to provide good surrogate measures for MS evolution. An International consensus conference of the

Table 4 Schematic Characterization of Magnetic Resonance Imaging-Monitored Trials of Multiple Sclerosis

<table>
<thead>
<tr>
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<th>Exploratory trials (phase II)</th>
<th>Definitive trials (phase III)</th>
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<tbody>
<tr>
<td>Outcome measure</td>
<td>Primary</td>
<td>Secondary</td>
</tr>
<tr>
<td>Sampling frequency</td>
<td>Monthly</td>
<td>Yearly</td>
</tr>
<tr>
<td>Main carrier</td>
<td>Gadolinium T1</td>
<td>Unenhanced T2</td>
</tr>
<tr>
<td>Principal target</td>
<td>Individual lesion</td>
<td>Lesion load</td>
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<tr>
<td>Method of detection</td>
<td>Visual</td>
<td>Computer assisted</td>
</tr>
<tr>
<td>Required resolution</td>
<td>Contrast</td>
<td>Spatial</td>
</tr>
<tr>
<td>Outcome parameter</td>
<td>Number (volume) of lesion</td>
<td>Change in lesion volume</td>
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</tbody>
</table>

Source: From Ref. 239.
White Matter Study Group of the International Society for MR in Medicine has indeed recommended the use of MT MRI in the context of MS clinical trials as an adjunctive outcome measure (260). Several recent MS clinical trials have already incorporated MT MRI, with a view to assess the impact of treatment on demyelination and axonal loss. To our knowledge, MT MRI has been used in phase II and phase III trials for RRMS (injectable and oral IFN beta-1a, IFN beta-1b, oral glatiramer acetate) and SPMS (IFN beta-1b and immunoglobulins). In these trials, MT MRI acquisition has been limited to highly specialized MR centers and only subgroups of patients (about 50–100 per trial) have been studied using MT MRI. The results of two of these trials have been published and have shown a lack of an effect of IFN beta-1b (261) and intravenous immunoglobulins (262) on MT MRI-derived quantities of the whole brain tissue and NAWM from patients with SPMS. Few studies were conducted at single centers with small numbers of patients (263–265) and have achieved conflicting results. Two of these studies have shown that treatment with IFN beta-1a (264) or IFN beta-1b (265) favorably modifies the recovery of MTR values which follows the ceasement of enhancement in newly formed lesions from RRMS patients. On the contrary, Richert et al. (265) did not find any significant difference in the MTR values of NAWM before and during IFN beta-1b therapy, as well as in the parameters derived from whole brain MTR histograms (263).

Only a few studies have been conducted to evaluate the effect of disease-modifying MS treatments on 1H-MRS-derived parameters (266–269). Using monthly 1H-MRS scans, Sarchielli et al. (266) found that treatment with IFN beta-1a has an impact on Cho peaks in spectra of lesions from RRMS patients, suggesting an increase in lesion membrane turnover during the first period of treatment. Narayanan et al. (267) found increased NAA levels in a small group of RRMS patients after one year of treatment with IFN beta-1b, suggesting a potential effect of treatment in preventing chronic, sublethal axonal injury. Schubert et al. (268) showed a stability of metabolite concentration over time in patients with RRMS treated with IFN beta-1b. More recently, Parry et al. (269) monitored with serial single-voxel 1H-MRS 11 patients treated with various formulations of IFN beta and found that the central white matter NAA/Cr ratio continued to decrease over the follow-up, suggesting that reduction of new inflammatory activity with IFN beta does not invariably halt progression of axonal injury.

CONCLUSIONS

Although cMRI has improved dramatically our ability to diagnose MS and to monitor treatment efficacy, it provides only limited pieces of information about MS pathology in terms of both accuracy and specificity. This suggests that the cMRI should not be used to establish long-term prognosis of individual MS patients (treated or untreated) and that the ability of a given treatment to modify metrics derived from cMRI does not mean necessarily that the treatment will be able to modify favorably the clinical course of the disease. This limitation may be overcome by the application of nonconventional MRI techniques, which should be used to define new MRI markers of MS evolution. Ideally, these new MRI markers should be quantitative, provide information about the most destructive aspects of MS pathology, and be derived from (at least) the entire brain. None of the MRI techniques taken in isolation is able to provide a complete picture of the complexity of the MS process and this should call for the definition of aggregates of MRI quantities, thought to reflect different aspects.
of MS pathology, to improve our ability to monitor the disease (171,270). Moreover, metrics derived from MT MRI, DW MRI, and $^1$H-MRS should be increasingly used to monitor MS evolution, either natural or modified by treatment. At present, longitudinal natural history data collected in large samples of MS patients (especially in those at the earliest clinical stage of the disease) using these MR techniques are needed to gain additional insight into disease pathophysiology and to define the role of modern MR technologies in the assessment of MS. Finally, in the evaluation of the relationship between clinical and MRI markers of disease evolution, the presence and efficacy of functional cortical changes should also be considered.

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Multiple Sclerosis Biomarkers

P. K. Coyle

Department of Neurology, School of Medicine, State University of New York at Stony Brook, Stony Brook, New York, U.S.A.

INTRODUCTION

Definition

Biomarker refers to an objective characteristic that can be evaluated and measured. It may reflect a normal biological process, a pathogenic process, or a response to therapy. In a recent review, different levels of biomarkers and endpoints were defined (Table 1) (1). One type of biomarker, previously termed surrogate marker, is now referred to as surrogate endpoint. The term surrogate indicates a biomarker with excellent clinical correlation, which provides reliable information more rapidly than clinical assessment and follow-up. The value of established biomarkers to diagnose and manage human diseases is unquestioned. The difficulty is in the identification and validation processes.

Role in Multiple Sclerosis

After trauma, multiple sclerosis (MS) is the major neurologic disease of young adults. MS affects at least 400,000 Americans, and up to two million people worldwide (2). Patient numbers appear to be increasing, not just within industrialized countries and Caucasian populations, but also in underdeveloped parts of the world and noncaucasian races (3).

MS biomarkers would be advantageous for many reasons, but none are established at this time. Several neuroimaging measures come close and additional novel imaging techniques are under study for validation and standardization. Certain features complicate biomarker development in MS. First, there are several disease subtypes characterized by relapsing or progressive courses. Despite distinct clinical and laboratory features based on group analysis, which suggest basic biologic differences, no biomarkers have been identified for these clinical subtypes. Second, MS is unlikely to be a single disease entity. It seems to encompass a spectrum of heterogeneous disorders which produce a similar clinical picture. It involves diverse pathologies (inflammation, demyelination, remyelination, axon injury and loss, oligodendrocyte and neuron loss, astrocyte gliosis) and damage mechanisms. In fact, four distinct immunopathologic patterns within acute plaques have been described (4). Although various processes contribute to disease disability, their contributions are likely to vary within distinct subpopulations. Principal damage mechanisms in MS
may also change over time. To date, there are no validated biomarkers for these varying pathologic processes and disease mechanisms. Third, MS is recognized as variable and unpredictable. No two patients are quite alike. Central nervous system (CNS) damage remains occult for a long time so that clinical evaluation does not assess disease activity status very well. This is especially true for the early disease phases. Frequent magnetic resonance imaging (MRI) studies indicate that 80% to 90% of new brain MRI lesions are not associated with definable relapse (5). Experimentally, global disease measures (brain and cervical spinal cord atrophy, whole brain N-acetyl aspartate on MR spectroscopy, magnetic transfer and diffusion tensor histograms) detect extensive but subtle abnormalities in normal appearing brain tissue in addition to the macroscopic plaques. This occurs even in early disease stages. The inability to evaluate the true extent of injury in daily practice makes accurate prognosis difficult. A prognostic biomarker would address this issue, but may be difficult to establish, since studies which use clinical attacks alone to determine active versus stable disease may not be valid. Fourth, it would be helpful to have biomarkers to guide drug therapy choice and judge treatment response. This is likely to be affected by multiple factors, including genetic background, host immune system, and disease stage. Finally, there is no definitive diagnostic test for MS. Diagnosis is based on a set of core clinical principles, supported by laboratory testing which typically includes appropriate blood work, MRI, cerebrospinal fluid (CSF) analysis, and sometimes evoked potential testing. With a misdiagnosis rate as high as 5% to 10%, a reliable diagnostic biomarker would be a major advance. All these features of MS highlight the advantage of biomarkers for diagnosis, measuring distinct damage mechanisms, identifying prognosis, and evaluating response to the MS disease modifying therapies (Table 2).

The need for MS biomarkers was highlighted at a recent National MS Society sponsored meeting on clinical trials. The meeting focused on how, with a shrinking population of treatment naïve patients, future MS trials could be conducted (6). Attendees endorsed the establishment of biomarkers to detect therapeutic benefits quickly, as well as the establishment of MRI markers that could substitute for clinical outcome measures. The next generation of therapeutic trials will focus on neuroprotection and CNS repair strategies to affect neurodegeneration and reverse disability. This will require novel biomarkers to measure features such as remyelination, axon and neuron integrity, microglial and endothelial activation, and astrogliosis and oligodendrocyte survival and repair.

<table>
<thead>
<tr>
<th>Table 1 Classification of Biomarkers and Endpoints</th>
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<tr>
<td><strong>Term</strong></td>
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<tr>
<td>Type 0 biomarker</td>
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<tr>
<td>Type 1 biomarker</td>
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<tr>
<td>Surrogate endpoint</td>
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<tr>
<td>Intermediate clinical endpoint</td>
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<td>Ultimate clinical outcome</td>
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*Source: From Ref. 1.*
Any valid and useful biomarker assay should meet key requirements (Table 3) (1,7,8). Potential MS biomarkers have additional considerations. One is a timing issue. The relevance of a given biomarker may differ depending on disease stage. MS has inflammatory and degenerative components. Inflammation, which corresponds to the relapsing phase of the disease, is maximum in the beginning and falls over time. Neurodegeneration, which corresponds to the progressive phase, is present at all timepoints but is unmasked late. These components are likely to have distinct biomarkers. MS involves simultaneous destructive and repair procedures that may complicate biomarker interpretation. A final consideration involves the type and source of biomarker. MS is an organ-specific disease, with pathology confined to the CNS. The only consistent systemic abnormality is immune system activation. Blood and urine, two potential sources for biomarker analysis, are distant from the site of the disease pathology. CSF is much closer, and is in part formed by CNS extracellular fluid, but involves a somewhat invasive lumbar puncture. Lumbar CSF does not always duplicate CSF collected closer to the brain.

There is an ever-growing literature on potential MS biomarkers. When reviewing this literature, it is important to keep in mind that single biomarker measurements are misleading when values show marked fluctuations. Many studies are cross-sectional, when in reality longitudinal studies would be more informative. Correlations based on group analysis are not necessarily meaningful for individuals, particularly when group values overlap. Finally, ideal biomarkers are unique to the disease and not influenced by other intrinsic and extrinsic factors. It is rare to find such disease-specific markers.

### Table 2  Potential Biomarkers in Multiple Sclerosis

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Goal</th>
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<tr>
<td>Diagnostic</td>
<td>Early diagnosis</td>
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<tr>
<td></td>
<td>To prevent misdiagnosis (5–10%)</td>
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<tr>
<td>Prognostic</td>
<td>To guide therapeutic selection</td>
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<tr>
<td></td>
<td>To counsel patient</td>
</tr>
<tr>
<td>Disease activity</td>
<td>To guide therapy decisions</td>
</tr>
<tr>
<td>Therapeutic response</td>
<td>To guide therapeutic changes</td>
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</table>

### Optimal Features/Special Considerations

Any valid and useful biomarker assay should meet key requirements (Table 3) (1,7,8). Potential MS biomarkers have additional considerations. One is a timing issue. The relevance of a given biomarker may differ depending on disease stage. MS has inflammatory and degenerative components. Inflammation, which corresponds to the relapsing phase of the disease, is maximum in the beginning and falls over time. Neurodegeneration, which corresponds to the progressive phase, is present at all timepoints but is unmasked late. These components are likely to have distinct biomarkers. MS involves simultaneous destructive and repair procedures that may complicate biomarker interpretation. A final consideration involves the type and source of biomarker. MS is an organ-specific disease, with pathology confined to the CNS. The only consistent systemic abnormality is immune system activation. Blood and urine, two potential sources for biomarker analysis, are distant from the site of the disease pathology. CSF is much closer, and is in part formed by CNS extracellular fluid, but involves a somewhat invasive lumbar puncture. Lumbar CSF does not always duplicate CSF collected closer to the brain.

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### Table 3  Optimal Features for a Biomarker Assay

<table>
<thead>
<tr>
<th>Feature</th>
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<tr>
<td>Reliable assay</td>
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<tr>
<td>Reproducible</td>
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<tr>
<td>Noninvasive</td>
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<tr>
<td>Simple to perform and interpret</td>
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<tr>
<td>Inexpensive</td>
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<tr>
<td>Detects fundamental disease feature</td>
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<tr>
<td>Validated in pathologic studies</td>
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<tr>
<td>High sensitivity and specificity</td>
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Source: From Refs. 1, 7, 8.
Body Fluids

Blood

Blood is an attractive fluid for a biomarker because it is relatively simple to collect. One can study the cells or soluble factors present in plasma or serum. However, blood is far removed from the site of disease pathology. Multiple systemic as well as organ-specific intrinsic and extrinsic processes can influence blood findings. Blood has diverse components, and there may be inhibitors present to interfere with a given assay. There are normal fluctuations in many factors associated with circadian rhythms that need to be accounted for. Finally, certain factors may be affected by handling and by freeze–thaw procedures.

Cerebrospinal Fluid

CSF is generated by choroid plexus and extracellular CNS fluid. CSF analysis can include cell and soluble components. The advantage of CSF as a body fluid source is that it is much closer to the tissue pathology in MS, so that findings are more likely to be relevant. It requires a mildly invasive procedure (lumbar puncture) so that repetitive sampling is not practical. There may be a slight cerebrocaudal axis gradient for certain factors, but this is not typically marked.

Urine

Urine is relatively easy to collect and will be enriched for excreted markers, particularly those with minimal tubular reabsorption (8). Marker concentration is affected by urine output over 24 hours, which is highly variable. Twenty-four hours sampling eliminates concerns about diurnal variation, but is very cumbersome. Creatinine is often used as an internal reference, with urine values expressed as a ratio. Albumin is used as a reference for peptides or proteins. Timing of sampling may be a factor so that first morning urines are often preferred. Urinary tract infection affects urine components and should be excluded. Urine is also influenced by systemic factors (9).

Mucosal Fluids

Mucosal fluids involve tears, saliva, breast milk, bronchial secretions, and gastrointestinal fluids. Several of these fluids have been studied in MS in limited fashion (8). There are difficult sampling issues that make routine use of these fluids impractical at the current time.

POTENTIAL BIOMARKERS

A recent review of potential MS biomarkers divided them into several broad categories (Table 4) (1). The classification is somewhat arbitrary with a heavy emphasis on immunologic measures. This section gives a more limited review of candidate markers, and tries to highlight those which are most promising.

Cytokines

Cytokines are soluble hormones of the immune system with multiple host effects. The complex cytokine network includes antagonistic proinflammatory and regulatory
cytokines, their soluble and bound receptors, cytokine inhibitors, and chemotactic cytokines referred to as chemokines. Individual cytokines have many actions, so it is generally too simplistic to consider them in terms of being good or bad for MS. They have been attractive candidates for biomarkers because they are known to be involved in MS disease activity and damage. However, they are influenced by many factors, changes may be modest, and values often show significant inter- and even intraindividual variability. This limits cytokine measurement as a useful MS biomarker. There are several ways to evaluate cytokines: absolute levels at a single timepoint, cell production, or gene transcription. Each method has its strengths and weaknesses.

A number of early studies focused on the proinflammatory cytokines interferon γ (IFNγ), associated with relapse induction, and tumor necrosis factor (TNFα), associated with oligodendrocyte damage. There is conflicting data on whether TNFα expression is enhanced prior to relapses (10–12). In a study of 13 untreated MS patients followed over nine months, MRI lesion activity was associated with a transient decrease in circulating T-cells which produced IFNγ and interleukin-4 (IL-4) (13). IFNγ production was reported as increased prior to relapse (14), while other studies found no consistent change (10–12). More recently, there has been a focus on IL-12 and IL-10. IL-12 regulates cell-mediated responses and promotes IFNγ production. In one study, increased peripheral blood mononuclear cell IL-12 mRNA preceded clinical relapses, and was detected when active MRI lesions

Table 4  Proposed Multiple Sclerosis Biomarker Classification

<table>
<thead>
<tr>
<th>Immune system changes</th>
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<tbody>
<tr>
<td>Cytokines, cytokine receptors (IL-1, -2, -6, -10, -12, -18; TNFα; LT-α/β; CD25)</td>
</tr>
<tr>
<td>Chemokines, chemokine receptors (CCR5, CXCR3, CXCL10, CCR2/CC chemokines)</td>
</tr>
<tr>
<td>Antibodies (CSF IgG index, k light chain, oligoclonal bands, anti-MOG/MBP antibodies)</td>
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<tr>
<td>Complement (C3, C4, activated neo-C9; activation regulators CD35, CD59)</td>
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<tr>
<td>Adhesion molecules (E-selectin, L-selectin, ICAM-1, VCAM-1, CD31, LFA-1, VLA-4)</td>
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<tr>
<td>Antigen processing and presentation (CD40/CD40L, CD80, CD86, heat shock proteins)</td>
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<tr>
<td>Activation markers (CD26, CD30, CD71, perforin, CD134, osteopontin, MR-8,</td>
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<tr>
<td>MRP-16, neopterin, amyloid A protein, somatostatin)</td>
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<tr>
<td>Cell cycle apoptosis (Fas/CD95, Fas-L, FLIP, Bel-2, TRAIL)</td>
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<tr>
<td>Immune mediated neuroprotection (BDNF)</td>
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<tr>
<td>Cell subpopulations (NK cells, Vα24+ NK T-cells, CD4+/CD25 bright and IL-10</td>
</tr>
<tr>
<td>producing immunoregulatory T-cells, CSF cells, CD45RA−/RO+/CD4+ memory T-cells)</td>
</tr>
<tr>
<td>Functional immunologic assays (proliferation, cytokine secretion, cytotoxic assays)</td>
</tr>
<tr>
<td>Blood–brain barrier disruption (MMPs and their inhibitors)</td>
</tr>
<tr>
<td>Demyelination (MBP, MBP-like material, proteolytic enzymes, endogenous pentapeptide</td>
</tr>
<tr>
<td>QYNAD, gliotoxin)</td>
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<tr>
<td>Oxidative stress, excitotoxicity (NO and its stable metabolites, uric acid, isoproprane,</td>
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<tr>
<td>hypoxia-like tissue damage marker)</td>
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<tr>
<td>Axonal/neuronal damage (cytoskeletal proteins: actin, tubulin, neurofilaments tau)</td>
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<tr>
<td>Gliosis(GFAP, S-100)</td>
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<tr>
<td>Remyelination, repair (NCAM, CNTF, microtubule associated protein-2, exon 13; 14-3-3</td>
</tr>
<tr>
<td>protein, CPK-BB, peptidylglycine-amidating monoxygenase, neural specific enolase)</td>
</tr>
</tbody>
</table>

Abbreviations: IL, interleukin; TNF, tumor necrosis factor; CSF, cerebrospinal fluid; MOG, myelin oligo-dendrocyte protein; MBP, myelin basic protein; MMPs, matrix metatloproteinases; NCAM, neuracell adhesion molecule.  
Source: From Ref. 1.
developed in relapsing and SP patients (12). In other studies serum IL-12 was elevated in SPMS, and elevated IL-12p40 subunit was found in the CSF of relapsing patients with contrast lesion activity (16,17). Upregulation of IL-12 was noted in relapsing and SPMS patients, but not primary progressive (PP) MS patients (18).

IL-10 is produced by TH2 cells. It is an important regulatory cytokine which suppresses proinflammatory cytokine production. Serum IL-10 levels were reported to be decreased during active disease (19). In relapsing MS, IL-10 mRNA expression within peripheral mononuclear cells decreased before disease attacks and development of MRI lesions (12). In another study, IL-10 mRNA was suppressed in active relapsing and SPMS patients (20). Serum IL-10 levels were reported as decreased in relapsing MS patients, but increased as Gd+ MRI lesions resolved (21). In a study of SPMS, patients with high IL-10 levels had significantly less disability and T2 lesion load (22).

Both IL-12 and IL-10 have been evaluated as potential therapeutic response markers. Baseline IL-12 p35 mRNA levels were said to predict outcome in 81% of interferon β (IFNβ) treated patients. Patients with a good response had lower baseline IL-12 p35 mRNA expression in peripheral blood cells than those with a poor response (23). After initiation of IFNβ or glatiramer acetate (GA) therapy, patients were reported to show increased serum IL-10 levels and mRNA, along with decreased TNFα levels (24–26). In IFNβ treated patients, increases in CSF IL-10 levels were said to correlate with a good response to therapy (26). IFNβ therapy also led to an increased proportion of IL-10 secreting CD4+ T-cells (27).

Osteopontin is a T-cell cytokine also called early T-lymphocyte activation-1 factor. It plays an important role in both acute and chronic inflammation. Microarray analysis and high throughput cDNA sequencing indicate osteopontin as the most abundant cytokine encoding gene within MS plaques (28,29). In a study of 30 relapsing, 10 PP, 10 SPMS patients, and 10 healthy controls, plasma osteopontin levels were significantly elevated in the MS cohort. Relapsing MS patients showed higher levels during clinical attack (28). The authors concluded that increased osteopontin levels were associated with disease activity in relapsing MS. In a follow up longitudinal study of 10 patients, there was a trend for osteopontin levels to be associated with clinical relapses (30).

There has been an interest in chemokines in MS, since they are implicated in cell trafficking into the CNS. Chemokines, MCP-1 and IP-10, were evaluated in serum and CSF of acute and stable MS, as well as healthy and other disease controls (31). CSF MCP-1 was significantly lower in acute MS versus stable MS. When detected, serum and CSF IP-10 levels were significantly higher in acute MS. However, patients with HIV-1 associated dementia also showed high levels. Treatment with methylprednisolone or IFNβ1a did not influence serum chemokines levels. In a recent study, chemokine expression change with GA treatment. Th1 associated chemokine receptor expression (CXCR3, CXCR6, CCR5) were downregulated, while the lymph node homing CCR7 receptor was upregulated (32).

Immunologic patterns may be more valuable than single factors. A recent multiparametric analysis of mRNA for 25 cytokine network components in peripheral mononuclear cells was able to distinguish MS from controls, and PPMS versus relapsing MS (33).

**Costimulatory Molecules**

Costimulatory molecules provide the second signal for cell activation. They involve the B7 family (CD80, CD86), expressed on antigen presenting cells, and their
corresponding ligands on T-cells (CD28, CTLA-4, CD40L). Increased CD80+ B-cells were reported during periods of MS disease activity (34,35), while CD86+ monocytes were decreased (36).

Costimulatory molecule expression on CSF cells does not appear to be a useful biomarker. CD80 and CD86 expression were studied on CSF monocytes from patients with MS, optic neuritis, neurologic Lyme disease, viral meningoencephalitis, and noninflammatory diseases (37). CD86 expression predominated over CD80 in all groups. There was increased expression of CD80 in MS and especially optic neuritis patients with a very short disease duration, but not during relapse.

**Immunoglobulins**

Qualitative and quantitative immunoglobulin assays are used in CSF to aid diagnosis (38). Oligoclonal bands (OCBs) are the most specific CSF test for MS, but they can occur in any chronic infectious or inflammatory disorder, and rarely in normal individuals. CSF OCBs ultimately develop in over 95% of MS patients. They correlate with the presence of plasma cells within meninges and plaques. A recent prospective study evaluated a new assay to detect CSF OCBs using isoelectric focusing followed by IgG immunodetection with alkaline phosphatase-labeled anti-IgG antibody (39). Of 132 MS patients, 127 (96.2%) were OCB positive. Only 1 in 100 (1%) of noninflammatory neurologic diseases and 18 of 51 (35.3%) CNS infections were positive. None of 63 other inflammatory neurologic disease controls was positive. Most positive MS patients showed a pattern of more than two OCBs in CSF, with a polyclonal distribution in the paired serum sample. In contrast, 16 of 18 positive CNS infection cases showed OCBs in both CSF and serum, but with more than two additional bands in the CSF. If infections were excluded, sensitivity for MS was 96.2% and specificity was 99.5%. OCB negative patients are said to have a better prognosis, but the data to support this is limited (40–42). The other major diagnostic CSF immunoglobulin assay is intrathecal IgG production, typically demonstrated by an elevated IgG index. In a study of intrathecal IgG synthesis, numbers were higher in SPMS than relapsing or PPMS (43). A very high IgG index was associated with more rapid rate of disability.

There have been a few studies of CSF IgM in MS. CSF IgM OCBs, elevated IgM, and increased IgM index were more likely to be detected during acute relapses and with clinical disease activity (44–46). CSF IgM OCBs were reported to occur in 46.2% of 65 MS patients (47). These patients showed greater disability as measured by EDSS.

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There is limited data from an Italian group on serum autoantibodies, which reacted to a structure-based designed glycopeptide CSF114(Glc) in 37 MS patients (48). The isolated antibodies recognized myelin and oligodendrocyte antigens. Development of antibodies was said to parallel clinical and MRI activity, but patient numbers are limited and the assay has not been independently duplicated.

A recent review article suggested a composite serum antibody index as a disease marker for MS. Elevated IgG to MBP, Acinetobacter (a bacteria species), and neurofilament (MAN) was proposed as a MAN index to predict relapses and perhaps response to therapy (49).

Free kappa light chain detection has been suggested as a useful CSF diagnostic test, but does not seem to offer any value above OCB (50). It can be used to resolve equivocal OCB readings (38).
**Cell Subpopulations**

Because of accessibility, cell subpopulation studies have focused on blood. Peripheral cells show increased activation markers. In a prospective study of 40 untreated patients with relapsing and progressive MS followed for one year, changes in activated T-cell populations in the blood were correlated with clinical and MRI disease activity (51). In relapsing MS, increases in CD4+CD25+ cells correlated with clinical attacks, while increases in CD25+ and CD4+I3+ cells correlated with increased EDSS. Increases in CD4+CD26+ cells in relapsing patients, and increases in CD4+I3+ cells in SPMS patients, correlated with a simultaneous increase in Gd+ lesions. Increase in I3+ cells in SPMS correlated with a simultaneous increase in T2 lesion volume. In relapsing MS, increase in CD25+ cells correlated with subsequent increase in T2 lesion volume, while in progressive MS, increase in CD26+ and CD4+CD26+ cells correlated with increased lesion burden. Decrease in CD4+I3+ cells correlated with an increase in Gd+ lesions and more new Gd+ lesions. In contrast to these activation markers, changes in CD3+ and CD4+ T-cells did not correlate with clinical or MPI measures.

In a cross-sectional study, kinin B1 receptor mRNA transcripts and protein were significantly upregulated on circulating lymphocytes during active disease in relapsing and SP patients compared to stable MS and controls (52). In a follow up study examining serial blood samples from six relapsing MS patients, increase in the kinin B1 actin mRNA preceded or were simultaneous with increase in EDSS, clinical relapses, T2 lesion volume, and increased percentage of IL-2 receptor positive, CD4+ T-cells, CD26+, and MHC class II peripheral mononuclear cells. These are leukocyte activation markers (53). Increased kinin B1 actin mRNA did not correlate with Gd+ lesions. This somewhat puzzling lack of correlation was felt to reflect the small sample size and the limited number (N=15) of Gd+ lesions. B1 receptor mRNA levels were much lower and more stable in controls than in the MS group.

In a study of CD10 (neutral endopeptidase) and CD13 (aminopeptidase N) activation markers on peripheral mononuclear cells, both markers were significantly higher in acute relapsing and progressive MS compared to patients in remission and OND controls (54).

Treatment with GA induces a shift from Th1 to Th2 cells. GA reactive CD8+ T-cells expand, while GA reactive CD4+ T-cells diminish over time (55).

**Matrix Metalloproteinases**

Matrix metalloproteinases (MMPs) are zinc-based enzymes, which allow cells to migrate through extracellular matrix and basement membrane. MMP expression, as well as the ratio of MMP to tissue inhibitor of MMP (TIMP), have been proposed as blood biomarkers for disease subtype, activity, and response to therapy. MS plaques and lymphocytes contain elevated MMP-2,-7, and -9 (56). MMP-2 and MMP-7 mRNA expression were reported as increased in peripheral lymphocytes of relapsing MS, while only MMP-7 was increased in SPMS (57). Increased MMP-9 levels correlated with Gd+ lesion activity in relapsing MS (58–61). Serum MMP-9 to TIMP-1 ratio, but not MMP-2 to TIMP-2 ratio, predicted Gd+ lesion activity in SPMS (62). In other studies, MMP levels and mRNA were elevated in the blood of MS patients during acute relapses (63,58).

MMPs have also been evaluated as a treatment response marker, since down-regulation of MMPs is believed to be one of the mechanisms of action for IFNβ
in MS. Relapsing MS patients who responded to therapy, as measured by clinical attack and disability outcomes, showed significant reduction in MMP-7 and MMP-9 mRNA levels in peripheral blood lymphocytes (57). This reduction was not seen in SP patients. In another study, IFNβ1b therapy in relapsing MS was associated with decreased serum MMP-9 levels and increased intercellular adhesion molecule-1 (64). Degree of changes seemed to correlate with treatment response.

Oxidative Stress

Oxidative stress has been implicated as an important damage mechanism. Isoprostanes are formed within membranes, and then released in free form. These lipid peroxidation products measure free radical generation. Isoprostane 8-epi-prostaglandin-F2α, the major F2-isoprostane compound, was examined in CSF from definite and probable MS, and OND controls (65). Levels were highest in those with definite MS. Steroid therapy was associated with lower levels. For the entire MS group, there was a modest correlation between levels and EDSS disability.

Uric acid is an endogenous peroxynitrite scavenger. Mean serum uric acid level was reported as significantly lower in clinically active relapsing and SPMS patients versus inactive patients and healthy controls (66). Uric acid levels were inversely correlated with Gd+ lesion activity (67). A prospective study found levels lower during relapse compared to remission (67). However, another study did not confirm correlation between uric acid levels and disease activity (68). With regard to treatment effect, serum uric acid levels increased after six months of GA therapy (69). They temporarily increased (for one month) after a course of high dose steroids (70).

CSF nitric oxide metabolites were reported as increased in relapsing and PPMS patients compared to controls (71). Patients with mild disability showed higher levels than those with severe disability. Metabolite levels correlated with Gd+ lesion volume. Over a three year follow up, higher levels were associated with development of greater disability and MRI lesion load. In another study, nitric oxide production by peripheral blood leukocytes was reported higher in MS cells versus control cells (72).

Myelin Components

MS is a demyelinating disease. There has been great interest in attempting to document a primary myelin target in MS such as myelin basic protein (MBP), myelin oligodendrocyte protein (MOG), or proteolipid protein. MBP, or MBP-like material, can be found in CSF using radioimmunoassay or enzyme linked immunosorbent assay (73,74). Levels are high during relapse, then fall and become undetectable. This was initially suggested as a disease activity marker rather than a diagnostic marker, since any destructive disorder can cause increased CSF MBP. However, it is not always detected during relapse, so that CSF MBP has not emerged as a practical biomarker for either diagnosis or disease activity. Although MBP documents myelin injury, it does not necessarily indicate a demyelinating disorder.

One research group reported detection of urinary MBP-like material, with the major component p-cresol-sulfate. Urinary MBP was particularly elevated in SPMS, appeared to correlate with transition from relapsing to SPMS, and with MRI parameters (T2 lesion number and volume; T1 hypointense lesion volume) (75–77). The assay is cumbersome and has not been duplicated in any other laboratory.

Antibodies to myelin components have also been studied. Intrathecal CSF anti-MBP IgM was associated with a more benign course (fewer attacks and less disability) over a mean follow up of 2.7 years (78). Of 66 relapsing patients, 23
(33.8%) had anti-MBP IgM. Lymphocytic meningitis patients also showed elevated levels. In a study of clinically isolated syndrome patients with a first attack of MS, serum IgM to MOG and MBP predicted shorter time to the next attack (79). This finding has not been duplicated, and the investigators may have detected cross-reactive rather than true antibodies to myelin components. In a recent study of 26 CIS patients, intrathecal IgM synthesis directed against myelin lipids correlated with more rapid second clinical attack (80).

Axonal/Neuronal Injury and Gliosis Markers

MS is not just an inflammatory and demyelinating process, but also involves abnormalities of axons, neurons, and glial cells. The neurodegenerative phase of MS injures axons and neurons. This appears to be the anatomic substrate of permanent disability. Biomarkers of axonal/neuronal injury would be useful not only prognostically, but also to follow the degenerative phase. Axon damage releases components such as neurofilament chains and tau protein into CSF. These components have been proposed as biomarkers for axon damage (Table 5) (81).

Neurofilaments are the major axonal cytoskeleton proteins (81). They consist of a triplet protein, including a neurofilament light chain (NFL), intermediate chain (NFM), and heavy chain (NFH). NFL forms a backbone; NFM and NFH polymerize to create neurofilaments. Neurofilaments are phosphorylated to varying degrees, with increases in their diameter. Axonal transection results in neurofilament breakdown, with release into CSF. A number of studies have examined NFL and NFH, as well as antibodies to these proteins. In a study of 34 MS patients followed for three years, NFH levels at follow up correlated with two clinical markers of disability, the nine hole peg test and EDSS (82). Three relapsing patients who converted to SP disease during the observation period had a higher median NFH level than those who did not convert. In earlier studies, CSF NFL levels correlated with EDSS in both progressive (83) and relapsing MS patients (84). In a recent review of the literature, CSF NFL levels were increased in MS patients compared to controls, and rose within several weeks of a relapse (81). Autoantibodies to NFL and NFH have been reported in CSF (85,86). The autoantibody index (CSF to serum ratio, divided by the albumin ratio) was found to correlate with brain atrophy measures.

Tau is a phosphorylated microtubule-associated protein primarily localized to neuronal axons. It promotes polymerization and stability of microtubules, and is

<table>
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<th>Table 5</th>
<th>Axon Injury Markers</th>
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<tr>
<td><strong>Cytoskeleton</strong></td>
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<tr>
<td>NFL chain, antibodies to NFL</td>
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<tr>
<td>NFH, antibodies to NFH</td>
<td></td>
</tr>
<tr>
<td>Actin and tubulin, antibodies to actin and tubulin</td>
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<tr>
<td><strong>Tau</strong></td>
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<tr>
<td><strong>Membrane markers</strong></td>
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</tr>
<tr>
<td>Apolipoprotein E</td>
<td></td>
</tr>
<tr>
<td>24 S-hydroxycholesterol</td>
<td></td>
</tr>
<tr>
<td><strong>Other markers</strong></td>
<td></td>
</tr>
<tr>
<td>14-3-3 protein</td>
<td></td>
</tr>
<tr>
<td>Neuron specific enolase</td>
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</tbody>
</table>

*Abbreviations: NFL, neurofilament light chain; NFH, neurofilament heavy chain.*

*Source: From Ref. 81.*
critical for intraneuronal transport. CSF tau levels were reported to be significantly elevated in MS versus control patients. Levels were higher in PP and SPMS compared to relapsing MS (87). Tau levels correlated with IgG index only in relapsing patients. The authors suggested that axon damage in the relapsing phase of MS was associated with the strength of the inflammatory response, but this no longer held true in the progressive phase. In another study of CSF tau in 17 MS patients, the levels were significantly higher during acute relapses (88). This result was not confirmed in a subsequent study of 20 MS patients, including 17 in relapse, and 32 matched controls (89). Tau was not elevated in CSF from the MS group compared to controls, even during acute disease attacks. In a recent study of CSF tau levels in relapsing MS (N = 35), SPMS (8), PPMS (9), CIS patients (50) and healthy controls (46), levels were significantly elevated in MS patients but did not discriminate between subtypes (90). The CIS group had the highest levels. CSF tau levels were significantly elevated in MS patients with Gd+ MRI lesions. There was a tendency for higher levels in patients with greater intrathecal IgG production. The authors interpreted these associations as supporting link between axonal damage and inflammatory activity. In a longitudinal study of 32 patients followed up to three years, elevated baseline CSF tau levels were associated with more rapid decline (91). Serum tau levels (which are typically tenfold less than CSF levels) have not been extensively studied.

There have been reports that increased CSF actin and tubulin in progressive MS correlated with EDSS (83). Other potential axonal/neuronal markers which have been looked at are the 14-3-3 protein, neuron specific enolene (NSE), and the brain specific cholesterol metabolite 24S-hydroxycholesterol. This last component is the only neuronal marker with promising results in blood (81). In a small study of 38 CIS patients, 5 (13%) had positive 14-3-3 protein in CSF (92). This neuronal injury marker predicted shorter time to second clinical attack. These patients had more relapses and developed greater disability over an average follow up of 27.3 months.

Glial markers have also been looked at. Glial fibrillary acidic protein (GFAP) is the intermediate filament of fibrillary astrocytes, and a key component of astroglisis. S100B is a calcium binding protein expressed in astroglial cells. CSF was examined for NFL and GFAP in 99 MS patients and 25 controls (93). Patients were followed up to 10 years afterwards. MS spinal fluid had elevated levels of both markers compared to controls. NFL was increased particularly during relapses and in progressive (especially SP) MS patients. Both CSF markers correlated with disability, but this was especially true for the axon injury marker.

In an earlier study, a series of CNS proteins (NFL, GFAP, S100B, NSE) were examined in the CSF of 66 MS patients and 50 controls (94). NSE is an energy metabolic protein of neural cell bodies. NFL was increased in all MS samples, particularly during relapse. GFAP was highest in SPMS and correlated with EDSS disability. In contrast, S100B and NSE levels were not different between MS and controls.

Apolipoprotein E (APO-E) is mainly produced and localized to astrocytes in the CNS. During injury it can be found in neurons as well. The APO-E4 allele has been associated with more severe MS in several studies. APO-E4+ MS patients were reported to have a higher relapse rate, greater brain atrophy, and greater development of more destructive (T1) lesions (95,96).

Miscellaneous

Transferrin is an iron-binding beta globulin glycoprotein synthesized predominantly in the liver. It plays a key role in iron metabolism. CSF transferrin appears to be an
inflammatory marker which reflects not just serum leakage but also production by CNS components (capillary endothelium, ependyma, oligodendrocytes) and inflammatory cells. In a study of paired CSF and serum from 51 MS patients, serum transferrin was lowest in PPMS, while the CSF to serum quotient (ratio $\times 10^3$) and index were highest in PPMS (97). The transferrin quotient was lower in stable relapsing MS patients versus those experiencing relapse. CSF transferrin and quotient were lower in early and younger MS patients. The authors proposed that evaluation of CSF and serum transferrin might be helpful for subtype differentiation, but indicated that it might be more meaningful in the context of a CSF protein panel for MS. In another study, serum iron, ferritin, transferrin, and soluble transferrin receptor were studied in 27 active or stable MS patients and 40 controls (98). There were no differences in hemoglobin, iron, and transferrin levels. However, soluble transferrin receptor levels were significantly higher in active relapsing and progressive patients. Ferritin levels were elevated in active progressive patients. Progressive patients compared to relapsing patients had higher ferritin levels. The authors interpreted these results to suggest that increased iron turnover (manifested by increased serum soluble transferrin receptor and ferritin) was associated with active disease.

Neural cell adhesion molecule (NCAM) is a member of the immunoglobulin gene superfamily involved in myelination and remyelination. Several different isoforms are expressed by glia, precursor cells, and myelin sheaths. CSF NCAM levels were reported to increase in MS patients following steroid therapy and coincident with clinical improvement following relapse (99). Steroid treatment alone was not sufficient to increase CSF NCAM.

Apoptosis markers have been studied in MS. A reduced ratio of pro- to anti-apoptosis Bcl-2 components was noted in peripheral blood lymphocytes from active versus stable MS (100). Survivin, an anti-apoptosis protein, was overexpressed by mitogen stimulated T-cells from active versus stable patients (101). Patients who responded to IFNβ therapy reduced expression of survivin (102). In a study of CSF from PPMS and other noninflammatory neurological diseases, CSF was added to cultured neurons for eight days (103). Neurons exposed to CSF from worsening PPMS showed apoptosis. This was not due to TNFα and suggested another soluble factor. However, these studies were only conducted on a total of 11 patients.

Endothelial cells which are activated, or undergoing programmed cell death, release endothelial microparticles that can be detected in plasma using a flow cytometric assay (104). These microparticles stain for platelet endothelial cell adhesion molecule-1 (Pecam-1/CD31) or vitronectin receptor (CD51). Patients in acute relapse had a 2.85-fold increase in CD31+ microparticles, which correlated with Gd+ lesions. This was consistent with a marker for acute injury. Compared to controls, CD51+ microparticles were elevated in MS patients whether they were in relapse or remission, consistent with chronic injury. The authors interpreted this as evidence for endothelial dysfunction during acute disease attacks, and that CD31+ endothelial microparticles might be a useful marker of disease activity.

The MS lesion project has suggested four distinct immunopathologies for acute plaques (4). Pattern III involves oligodendrocyte dystrophy with apoptosis, and mimics the myelin destruction that occurs with acute ischemia. In this pathology, there is nuclear expression of hypoxia-inducible factor-1α. It is also highly expressed within ischemic brain lesions. A monoclonal antibody (D-118) against the nucleocapsid protein of canine distemper virus detected a HIF-1α correlated phosphorylated epitope shed into CSF (105). The authors suggested that the CSF epitope could be a useful biomarker for this MS subset.
Platelet activating factor (PAF), a phospholipid inflammation mediator, has been proposed as a blood–brain barrier injury marker. In a study of 11 relapsing, 9 SPMS patients, and 6 control subjects, CSF and plasma PAF levels were significantly increased in MS patients compared to controls (106). Relapsing MS showed higher levels than SPMS. Levels correlated with the number of Gd+ lesions, but did not correlate with EDSS.

In a cross-sectional study of CSF soluble adhesion molecules in relapsing PP, and SPMS, only intrathecal production of soluble VCAM-1 was noted, and only in relapsing MS (107).

C reactive protein, (CRP) an acute phase reactant, was measured serially in MS patients participating in a SC IFNβ1a trial (108). CRP rose during relapses and fell with IFNβ therapy. Higher levels during the first year of therapy correlated with later disability. A purported blood test for diagnosis of MS used matrix-assisted laser desorption/ionization time of flight mass spectroscopy to examine 25 relapsing MS patients and 25 healthy controls (109). The authors identified three markers for MS. This study awaits validation.

**Novel Techniques**

New technology is being used to carry out large scale analysis of mRNA transcripts and autoantibody responses within MS tissues. These techniques include large scale sequencing from cDNA libraries, oligonucleotide microarrays, single-nucleotide polymorphisms, and expressed sequence tag (110). One can examine upregulated and downregulated genes in MS versus controls, as well as changes that occur with drug therapy. This could lead to genetic profiles to choose specific drug therapy, and to determine response to therapy. Proteomics allows the large scale analysis of autoantibodies. This is being developed for therapeutic intervention, such as DNA vaccines to tolerize or remove any damaging humoral response.

Optical coherence tomography (OCT) provides a morphologic evaluation of the retinal nerve fiber layer thickness. OCT indicates reduced thickness in optic neuritis patients for example, and has been proposed as a marker for occult axon injury in MS (111).

**NEUROIMAGING**

MRI is recognized as the best current biologic marker of disease activity in relapsing and to a lesser extent in SPMS (112). Evaluation of Gd+ lesions is often a primary outcome in preliminary trials of a new agent for relapsing MS, to determine whether it should be studied further. MRI is routinely used for diagnosis but there are no pathognomonic lesion features. Lesions which show perpendicular orientation to the ventricles, multiple and large (>3 mm) lesions, certain locations (corpus callosum, juxtacortical, infratentorial, spinal cord), and enhancing lesions (especially open ring enhancement) favor a diagnosis of MS. MRI is somewhat helpful for prognosis in first attack and early relapsing MS, where seeing many large lesions, a high T1 to T2 ratio, and obvious atrophy suggest a worse prognosis. MRI is used variably to judge treatment response. Significant increase in T2 lesion burden while on any therapy, or persistent Gd+ lesion activity on IFNβ suggest poor response. No MRI finding is yet established as a true MS biomarker. The one that is most likely to be accepted is Gd+ lesion activity as a marker of clinical relapse. A number of
novel MRI techniques can measure global CNS injury and can detect abnormalities outside macroscopic plaques (Table 6). These techniques may be established as MS biomarkers in the future.

CONCLUSION

The need for MS biomarkers is clear, and their development will be a priority over the next few years. Established biomarkers would be helpful for diagnosis, prognosis, determination of disease activity, and response to therapy. They could be useful to identify and track distinct pathologies and damage mechanisms, and to better understand MS heterogeneity. At this point there is no established biomarker. Gd+ lesion activity is most likely to be accepted in the near future as a marker for clinical disease attack. Further biomarkers, perhaps in the form of a validated neuroimaging battery, are likely to be developed. Whether any blood or CSF biomarker will be established for MS is not yet clear. However, in the next few years pharmacogenomics is likely to begin to provide genetic biomarkers for prognosis and treatment response.

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INTRODUCTION

Evoked potentials (EPs) are electrical potentials, generated by the nervous system, that are evoked by certain sensory stimuli. These tests have been used for the past 25 years by clinicians seeking to diagnose multiple sclerosis (MS). They have also been applied in research in the pathophysiology of demyelination and as an adjunct in MS therapeutic trials. EPs have found a permanent role in several diagnostic and research areas (1–5).

EPs are sensitive, objective, reproducible, and can be quantified easily to two to three significant figures. They can detect “silent lesions,” i.e., physiologic changes not accompanied by physical signs or localizing symptoms. Finding silent lesions can help diagnose MS by providing evidence of a second or third lesion. The tests are objective because they require no patient participation except for lying quietly or watching a video screen. A patient cannot alter the results. The reader scores the tests in a standard manner that leaves little room for subjective error. EPs are reproducible, yielding identical values as long as the conditions of the testing are well controlled. These tests can be quantified to two to three significant figures, aiding comparison of results to normal values. Quantified parametric measurements and statistics are also substantially more powerful tools than discontinuous categorical variables (e.g., mild/moderate/severe or better/worse/unchanged scoring) for evaluating scientific hypotheses.

EPs represent electrical potentials (voltages) that are evoked by brief sensory stimuli. Nerve volleys are conducted along the peripheral and central nervous system (CNS) pathways of the stimulated sensory modality. These signals are delayed or blocked when they cross through a demyelinated region. In classical demyelination, conduction delay occurs through the region of impairment up to complete conduction block across a demyelinated region (6–10). EPs generated beyond the demyelination site are abnormal because they are delayed, attenuated, or absent. With the knowledge of generator sites, the EP reader can determine the approximate nervous system level at which a delay or a block probably occurred. This allows a clinician to assess which parts of the nervous system have been impaired. However, EPs can only test a few selected nervous system pathways: the central visual pathways,
the brainstem auditory pathways, the lemniscal sensory pathways, and now the pyramidal pathways (11–13). Event-related potentials (ERP) also can measure the speed of cognitive processing, a technique that has been applied to MS. There is not yet any routine EP that can test spinothalamic or cerebellar pathways.

EPs have also been used in many other areas of neurologic practice beyond MS. Brainstem auditory evoked potentials (BAEPs) are used to screen for hearing impairment (14). All three sensory EP modalities (visual, auditory, and somatosensory) are used for evaluation of comatose patients, allowing quantified assessment of degree of impairment (15,16) and help in assessing locations of lesions. Hereditary-degenerative neurologic conditions are associated with specific patterns of changes in various EP peaks, which is sometimes useful in the diagnostic evaluation of these conditions (17). EPs can be monitored in the operating room, allowing for identification of nervous system impairment early enough to allow intervention to correct the impairment before it becomes permanent (18). Presence of normal EPs, despite severe symptoms, helps to confirm conversion hysteria or malingering. EPs also help to separate peripheral from central or spinal from intracranial localization for a variety of sensory disorders, analogous to the use of the tendon reflexes for separating central from peripheral motor pathway disorders.

VISUAL EVOKED POTENTIALS

Visual evoked potentials (VEPs) can be elicited with either a strobe flash or a checkerboard pattern reversal device. Use of the flash technique for MS was first described (19), but the pattern reversal VEP technique was found to be clearly more sensitive for detecting demyelinating lesions (20). Pattern reversal is typically a checkerboard of black and white squares, in which each white square becomes black and each black square becomes white twice each second. This can be accomplished on a television screen controlled by a small computer, or with a slide projector and galvanometer-mounted mirror. The subject is usually tested one eye at a time in a darkened room. Recordings are made over the occipital scalp. Measurements are made to the large positive electrical polarity peak, named P100, seen about 100 msec after each checkerboard reversal. About 100 separate stimulus presentations are performed, and their results averaged together help to eliminate random background “noise” such as electroencephalogram (EEG) and electrocardiogram (ECG). The P100 represents the culmination of a series of neurological events. These events begin with axon potentials conducted out of the eye along the optic nerve, across the chiasm, and up the optic tract to the lateral geniculate body. From there, the signal travels up the optic radiations, passing directly through the periventricular white matter for rather long distances, until it reaches occipital cortex. Substantial processing occurs at the occipital region for up to 50 msec after the arrival of initial impulse. Finally, a large electrical surface positive peak is generated from striate cortex and detectable at the occipital scalp as the P100 peak.

In MS patients, impairment may occur at several points along this pathway, not just at the optic nerve but also along the optic tract and especially in the periventricular white matter. Prechiasmatic lesions at the optic nerve can be separated from the postchiasmatic lesions by testing the two eyes separately. Interocular discrepancies in P100 latencies are usually attributed to lesions at the optic nerve for obvious anatomical reasons.

VEPs are more sensitive to demyelination than a careful clinical examination of visual function (21–23). Compared with careful neuro-ophthalmologic examination,
no exam abnormality was detectable when the VEP was normal (22). When the VEP was abnormal, various clinical examinations were often normal. For example, when the VEP was abnormal: 96% of patients had normal visual fields by confrontation, 55% had normal visual fields by formal testing, 74% had normal pupillary responses, 39% had normal appearance of the optic fundus, and there was no red color desaturation in 27% of patients tested carefully.

The checkerboard reversal pattern VEP technique is abnormal in almost all patients who have a clear history of optic neuritis (ON). In a summary of various reports in the medical literature (24–48), Chiappa (23) noted that about 90% of patients with ON showed abnormal pattern VEPs, with the percentage closer to 100% in many of the individual research reports. When there was no clinical evidence for ON the VEPs were still abnormal in 51% of 715 MS patients (Table 1).

VEPs tend to worsen monotonically. Once a lesion has occurred and the VEP has become delayed, only modest improvement occurs subsequently. A typical initial 30 msec delay improves gradually to a 16 msec delay over six months to a year (49,50). Otherwise, the delays are permanent (51). In this way, VEPs help to establish an episode of suspicious visual changes many years ago, which was indeed due to an episode of ON. This is, of course, of great value in diagnosis of MS. Patients presenting with single spinal cord or brainstem lesions are often referred for VEP studies to determine whether ON has occurred anytime in the past years. The finding of such a second, visual system lesion has helped substantially in establishing many a diagnosis of MS.

Other disorders can also affect VEP latencies. Some hereditary-degenerative neurologic conditions, e.g., Friedreich’s ataxia (17) and adrenoleukodystrophy (52), as well as B12 deficiency (53), neurosyphillis (54), and other disorders (55), can slow P100 latencies. Generally, these changes are mild to moderate bilateral P100 delays. A severe delay or a substantial interocular latency difference is usually due to MS. MS can sometimes cause mild to moderate symmetrical P100 delays by bilaterally symmetric demyelination. As such, the finding of mild to moderate bilaterally symmetrical P100 delays is considered confirmatory for an abnormality but nonspecific for the type of pathology. Overall, a VEP abnormality cannot be considered absolutely pathognomonic of MS. Clinical correlation is useful in each of these circumstances.

Table 1  Rates of Abnormalities for Evoked Potentials in Multiple Sclerosis: Aggregate Results of 26–31 Separate Research Series

<table>
<thead>
<tr>
<th></th>
<th>Pattern visual</th>
<th>Brainstem auditory</th>
<th>Somatosensory</th>
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<tbody>
<tr>
<td>Number of patients</td>
<td>1950</td>
<td>1006</td>
<td>1006</td>
</tr>
<tr>
<td>Number of research series</td>
<td>26</td>
<td>26</td>
<td>31</td>
</tr>
<tr>
<td>Rates of EP abnormality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definite MS (%)</td>
<td>85</td>
<td>67</td>
<td>77</td>
</tr>
<tr>
<td>Probable MS (%)</td>
<td>58</td>
<td>41</td>
<td>67</td>
</tr>
<tr>
<td>Possible MS (%)</td>
<td>37</td>
<td>30</td>
<td>49</td>
</tr>
<tr>
<td>Asymptomatic patients (%)</td>
<td>51</td>
<td>38</td>
<td>42</td>
</tr>
<tr>
<td>All patients (%)</td>
<td>63</td>
<td>46</td>
<td>58 (upper extremity) 76 (lower extremity)</td>
</tr>
</tbody>
</table>

**Abbreviations:** EP, evoked potential; MS, multiple sclerosis.

**Source:** From Ref. 23.
VEPs are commonly used to evaluate ON patients. Many idiopathic ON patients eventually develop MS (56,57). Initial VEPs were abnormal in (58) nearly all (50,59,60) eyes affected by ON. VEP was abnormal in the clinically unaffected eye in 25% to 35% of ON patients (58–60), and the presence of such a contralateral silent lesion greatly increases the chance that the patient will progress from idiopathic ON to MS.

The VEP is about twice as sensitive as magnetic resonance imaging (MRI) for detecting demyelinating lesions in the optic nerves, chiasm, and optic tracts (60–63). However, brain MRI is more sensitive than VEP of the unaffected eye (60,64) for searching generally any second lesion in ON patients. Brain MRI was abnormal, more often than VEP, in patients with early MS without ON (63,65,66). In ON, the length of the MRI-detected inflammation correlated with the severity of VEP delay (67). The degree of VEP delay did not predict the degree of MRI-detected long-term atrophy (68). The time course of resolution of gadolinium enhancement in ON parallels the time course of improvement in the VEP latency (69).

For patients with acute or chronic spinal cord lesions evaluated for a diagnosis of MS, multi-modality EPs had a higher yield of abnormalities (69% sensitivity) and a lower false positive rate (5% false positives for EPs compared to 9% for MRI). VEP alone, however, had only a modest to poor diagnostic yield (7–28%) or prediction of progression to MS for patients presenting initially with spinal lesions (70,71). VEPs are also helpful for clarifying the nature of signal enhancing MRI lesions by helping to separate MS from the dozen other causes of such lesions mimicking those of MS (72).

VEPs have been used to study the physiology of demyelination. The latencies and amplitudes can be affected by heat and medications that alter conduction across a demyelinated plaque. Heat alters VEPs (73–75) in a way similar to the clinical Uhthoff's phenomenon or the hot bath test. For the VEP, these effects can be quantified more precisely. Hyperventilation can improve the VEP, causing some improved amplitudes and even shorter latencies (76). This is in keeping with previous observations that hyperventilation, alkalosis, and hypocalcemia can bring about transient improvements in clinical deficits. The calcium channel blocker verapamil (77) and the potassium channel blocker 4-aminopyridine (78) can also substantially improve VEPs transiently in some patients.

The genetics of MS have been studied using VEPs (79). A small portion of asymptomatic first-degree relatives of MS patients were found to have mildly abnormal P100 VEP interocular latency asymmetries. This may be related to a genetic predisposition toward subclinical pathology such as plaques of edema without demyelination or with only subtle demyelination. For epidemiological reasons, most of these abnormalities are unlikely to develop into frank clinical MS. Some additional factor must be needed to change silent lesions into lesions associated with clinical MS. This may give some hints to the underlying multifactorial nature of MS.

Overall, checkerboard reversal pattern VEPs have proven themselves to be of substantial help in clinical evaluation of individual patients when MS is under consideration. The finding of abnormalities in these visual pathways is common, even in patients with no other clinical indications of central visual pathway impairment. VEPs are more sensitive than MRI in detecting ON. In typical clinical circumstances, these tests are useful in clarifying whether a previous visual event was ON or not, and in looking for visual pathway impairment in patients with single brainstem or spinal cord lesions.
BRAINSTEM AUDITORY EVOKED POTENTIALS

Signals from brainstem auditory pathway generators can be detected at the scalp. These signals represent activation of brainstem pathways after presentation of a 100 μsec click through earphones. Pathways involved are probably those associated with the ability to localize an auditory stimulus in space, rather than those used for speech or tone discrimination. These pathways lie exclusively in the pons and midbrain. These auditory EP tests are unable to detect lesions except for those located in the specific brainstem pathways tested here. They fail to detect lesions at or below the medulla, or at or above the thalamus. But these tests are so sensitive that they can pick up a delay of just a fraction of a millisecond when it does lie in the specific brainstem pathways tested.

The origins of the BAEP start at the eighth nerve, which is the generator of wave I. The presence of this wave I peripheral potential is valuable in assessing the click stimulus, which had been adequately processed by the cochlea and other peripheral portions of the auditory pathway. Of course, this wave I is almost universally normal in MS patients who have no additional specific ear related problems. The BAEP has four succeeding waves labeled II–V (Fig. 1). These arise from within the brainstem itself. Wave II is generated around the cochlear nucleus, at the caudal pons. Wave III arises around the superior olive and trapezoid body in the central pons. Waves IV and V probably arise from regions around the lateral lemniscus bilaterally, as each of these pathways travel rostrally toward inferior colliculus. CNS lesions can be localized by observing which wave was disrupted or delayed. The left–right laterality of lesions is more difficult to assess for the lower mid-brain or upper pons lesions. The laterality is fairly straightforward for lower pontine lesions. Impairment of the BAEP usually corresponds clinically to disruption of nuclei and pathways in the deep pons. Internuclear ophthalmoplegia is the most common clinical sign correlating with brainstem auditory EP abnormalities. Other brainstem signs have a lesser degree of correlation. Vertigo, dysarthria, and dysphagia have a rather mediocre to low correlation with abnormalities of these EPs (Table 2).

The typical abnormalities found in MS patients include a prolongation of waves II–V as determined by the I–V interpeak latencies and a loss of amplitude

![Figure 1](image-url) Brainstem auditory evoked potentials, identifying the five main peaks. Source: From Ref. 80.
of wave V, determined by V/I amplitude ratio and disappearance of V. Each of these types is almost equally common. Figure 2 shows examples of various degrees of BAEP abnormalities in MS patients.

Other types of neurologic disorders can also affect the BAEPs. These include damage from tumors (83,84) and ischemia (84), as well as changes associated with some hereditary degenerative neurological disorders (17). As such, BAEP abnormalities cannot be considered pathognomonic of MS. Rather, these abnormalities just indicate the presence of impairment at a pontine or lower midbrain level (85).

Chiappa (23) has summarized aggregate results from research reports (37,86–108) that included approximately 1000 MS patients (Table 2). Among these patients, 46% had abnormal BAEPs. Among patients having no history or physical signs of brainstem abnormalities, 38% had EP abnormalities, with abnormality rates in individual studies varying between 21% and 55%. The latter represent clinically silent lesions detected by these EP techniques.

BAEPs have repeatedly been found to be more sensitive to detecting pontine lesions than MRI tests (109–112). Brain MRI is more sensitive than BAEP to patients undergoing an evaluation to diagnose MS. Among three studies directly comparing the two tests, brain MRI was abnormal among 68% to 83% of patients, whereas BAEP was abnormal among 41% to 50% of patients (66,111,113).

Overall, BAEP seems an appropriate clinical tool to confirm that cranial nerve or other signs or symptoms are due to central, brainstem impairment as opposed to impairment along the peripheral pathways. The test is sensitive to impairment at pons and lower midbrain. For this specific purpose, it is probably more sensitive than brain MRI. For the general setting of evaluating possible MS patients, brain MRI has a higher yield of abnormality.

Table 2  Correlation Between Degree of Brainstem Auditory Evoked Potential Abnormality and Multiple Sclerosis Patient Signs and Symptoms

<table>
<thead>
<tr>
<th>Correlation with change in BAEPs</th>
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<tbody>
<tr>
<td><strong>History</strong></td>
<td></td>
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<tr>
<td>0.41</td>
<td>Diplopia</td>
</tr>
<tr>
<td>0.23</td>
<td>Dysphagia</td>
</tr>
<tr>
<td>0.16</td>
<td>Vertigo</td>
</tr>
<tr>
<td>0.12</td>
<td>Hearing impairment</td>
</tr>
<tr>
<td>0.10</td>
<td>Dysarthria</td>
</tr>
<tr>
<td>0.03</td>
<td>Facial sensory impairment</td>
</tr>
<tr>
<td><strong>Physical signs</strong></td>
<td></td>
</tr>
<tr>
<td>0.39</td>
<td>Ocular dysmetria or gaze paresis</td>
</tr>
<tr>
<td>0.32</td>
<td>Nystagmus</td>
</tr>
<tr>
<td>0.29</td>
<td>Facial weakness</td>
</tr>
<tr>
<td>0.25</td>
<td>Dysarthria</td>
</tr>
<tr>
<td>0.23</td>
<td>Facial sensory loss</td>
</tr>
<tr>
<td>0.21</td>
<td>Slow tongue movements</td>
</tr>
<tr>
<td>0.09</td>
<td>Other brainstem signs</td>
</tr>
<tr>
<td>0.4</td>
<td>Subjective hearing threshold</td>
</tr>
</tbody>
</table>

*Abbreviation: BAEPs, brainstem auditory evoked potentials.
Source: From Ref. 81.*
Somatosensory modality testing usually begins with a delivery of a brief electrical stimulus to the median nerve at the wrist or to the posterior tibial nerve at the ankle. Peripheral recordings are taken from electrodes located over the brachial plexus or the lumbar spinal cord. More rostral recording electrodes are placed over the cervical spinal cord and the scalp. Electrodes at these latter locations can detect electrical potentials signaling passage through progressively more rostral CNS tracts and

Figure 2  Examples of various degrees of abnormality in the brainstem auditory evoked potential test in multiple sclerosis. The upper evoked potential traces are less affected and the lower traces are more affected. Demyelination causes some prolongation of latencies, with loss of amplitude and eventual absence of peaks II–V. Source: From Ref. 81.

SOMATOSENSORY EVOKED POTENTIALS

Somatosensory modality testing usually begins with a delivery of a brief electrical stimulus to the median nerve at the wrist or to the posterior tibial nerve at the ankle. Peripheral recordings are taken from electrodes located over the brachial plexus or the lumbar spinal cord. More rostral recording electrodes are placed over the cervical spinal cord and the scalp. Electrodes at these latter locations can detect electrical potentials signaling passage through progressively more rostral CNS tracts and
Figure 3  Examples of the peaks seen in normal short latency (A) median nerve and (B) posterior tibial nerve somatosensory evoked potential testing. Negative potentials are upward deflections here. Recording sites EPI and EPc are at shoulders; C5Sp and T12 over the spine; PF, K, and IC at popliteal fossa, knee, and iliac crest; Ci, Cc, C'z, and Fz on scalp. The several standard peaks are identified here. Source: From Ref. 82.
nuclei. The pathways underlying somatosensory evoked potentials (SEPs) are the posterior columns, medial lemniscus, and internal capsule. At present, there are no routine clinical EPs for testing the spinothalamic pathways.

For median nerve EPs, the principle peaks detected are generated at the brachial plexus, mid-cervical cord, cervicomedullary junction, at or near the thalamus, and finally at the Rolandic fissure (Fig. 3). For the posterior tibial nerve EPs, reliable potentials are usually found only for the lumbar cord and Rolandic fissure generator sites. Occasionally, additional posterior tibial nerve SEP peaks can be detected over the rostral spinal cord or at brainstem levels, but these additional peaks are difficult to record in many normal subjects.

Comparison of the latencies and amplitudes of these various peaks can help the clinical reader to determine the anatomic level of disruption along these sensory pathways. In many circumstances, EPs can locate specific levels of disruption along these pathways. This is useful in MS where diagnosis requires finding lesions in separate locations. It is also useful in other neurologic evaluations in which approximate anatomic localization is valuable.

Chiappa (23) has summarized the aggregate results from clinical studies on abnormality rates for median nerve SEPs in MS (34–40,51,93–96,114–135). Median nerve EP abnormalities were seen in 42% of MS patients who had no signs or symptoms of sensory systems impairment and 75% of patients who did have signs or symptoms of appropriate sensory abnormalities. Posterior tibial nerve SEPs have revealed a slightly greater rate of finding clinically silent abnormalities (Table 1). The degree of SEP delays correlated with the expanded disability status scale (EDSS) (136).

A variety of neurologic disorders can affect SEPs. Peripheral neuropathy and other peripheral disorders can affect the peripheral conduction velocities. Fortunately, these peripheral effects can be removed from the analysis of CNS conduction by subtracting the latencies of the peripheral peaks seen over the brachial plexus or lumbar spinal cord. A variety of hereditary-degenerative neurologic conditions (17) can slow central conduction latencies in sensory pathways, as some acquired metabolic disorders such as B12 deficiency (137). Focal lesions due to ischemia, tumors, cervical myelopathy, and other focal disorders can also disrupt conduction along the central portions of the somatosensory pathways. As such, information from SEPs must be integrated with other clinical information in order to assess whether EP changes are due to MS or another neurologic disorder.

Brain MRI is more sensitive than either median nerve SEP or posterior tibial nerve SEP in MS. In a direct comparison in 46 suspected or confirmed MS patients, 25 (54%) had normal median nerve SEPs, 33 (72%) had abnormal posterior tibial nerve SEPs, whereas 34 (74%) had an abnormal brain MRI scan (113). In another study of 60 patients with definite, probable, or possible MS, 29 (48%) had abnormal median nerve SEPs, 37 (61%) had abnormal posterior tibial nerve SEPs, whereas 50 (83%) had an abnormal brain MRI (111). Similar results were seen for cervical MRI in 46 patients with spinal cord syndromes being evaluated for MS (71). In that study, 31/46 (67%) of patients had an abnormal cervical MRI, whereas 26/46 (57%) had abnormal SEPs.

Overall, SEPs provide a useful tool for detecting clinically silent lesions that contribute to the diagnosis of MS. They provide a sensitive way to assess the spinal cord pathways, that can complement other testing such as brain MRI.
MOTOR EPs

Neurons in cerebral cortex can be discharged by applying brief electrical stimulation at surgery. This can also be achieved through an intact skull. Considerable voltage is needed to drive electrical currents from the scalp through the skull to the cortex (e.g., 300–400 V). In patients who are awake such electrical stimulation is painful.

An ingenious solution to this painful situation has been devised. A powerful magnetic device held above the scalp can create a brief but extremely intense magnetic field. The skull is a resistor for electrical currents, but not for a magnetic field that passes unimpeded through the skull. According to the standard principles of electromagnetism, a fluctuating magnetic field invariably creates an electrical potential. The brief intense magnetic field above the scalp creates an electric current within the cerebral cortex strong enough to discharge the neurons. This technique can be focused at the cells in a particular one square centimeter under the location of the magnetic stimulator. Thus, various specific cortical regions can be stimulated by locating the magnetic stimulator coil precisely over the scalp.

This magnetic technique was popularized a decade ago by Barker et al. (138,139). Previously, investigators in MS and other neurological disorders had used transcranial electrical stimulation to study motor pathways (140,141). In either technique, recordings can be made at muscles or large peripheral nerves. Using the transcranial electrical technique, studies demonstrated marked prolongation of central motor conduction times in most of the MS patients tested (11–13). With the advent of magnetic cortical stimulation, the clinical feasibility of the technique improved greatly. Clinicians studying motor pathway stimulation generally use the magnetic techniques.

Several studies demonstrated a high rate of magnetic central motor conduction time delays in MS (142–150). The abnormality rate is even higher for lower extremity recording than upper extremity. Exercise can increase the abnormality rate (151). The technique is well suited for identifying silent lesions in MS patients. They prove an objective measure useful in MS therapeutic trials (152–154). There is a good correlation with MRI lesions in cervical pyramidal tracks (148), but a poor correlation with physical exam findings of weakness (147,155).

Magnetically motor evoked potentials (MEPs) were compared with multimodality evoked sensory potentials (VEP, BAEP, SEP) and also with MRI testing by Ravnborg et al. (146). In that study 68 patients clinically suspected of having MS were tested. Among the 40/68 (59%) patients eventually diagnosed as having MS, the MRI was positive in 88%, MEP 83%, VEP 67%, SEP 63%, and BAEP 42%. The MEP was abnormal also among one-third of the patients who eventually received other CNS diagnoses or no clear diagnosis. Among 10% of the MS patients, the MRI was normal but the neurophysiological tests were abnormal confirming a CNS disorder.

In another comparison of MEPs and sensory EPs in MS, Filippi et al. (156) found lower extremity SEPs to be abnormal often (75% of patients), followed by lower extremity MEPs (65%), VEPs (64%), upper extremity MEPs and SEPs (56% and 52%), and finally BAEPs (39%). They also noted that patients with chronic progressive MS had a high rate and greater degrees of EP abnormalities compared to patients with a more benign MS course. Others also reported worse MEPs among secondary progressive MS than relapsing–remitting MS (157), and that MEPs were abnormal more often than SEPs (154).
Central motor conduction tests can also demonstrate abnormalities in other neurological disorders. Slowed central motor conduction was found in motor neuron disease among 13/15 patients in one study (158) and 8/11 patients in another (159), whereas SEPs were normal. Patients with hereditary motor and sensory neuropathy (HMSN) had delayed central conduction when they had clinical signs of pyramidal disease, with degrees of delay differed in different specific subtypes of HMSN disorders, presumably corresponding to different specific pathophysiology (160).

Magnetically evoked central motor conduction tests should be considered a test available to search for clues in diagnosing MS, and in the differential diagnosis of other possible central motor disorders.

**EVENT-RELATED POTENTIALS**

The speed of cognitive processing can be measured by different kinds of evoked potentials referred to as event-related potentials (ERP). Several kinds are in use. These mark certain events in the brain’s internal recognition and decision-making processes. Several studies have applied these to MS.

The most common ERP uses the auditory oddball paradigm (161). A series of brief tones is presented, most at one pitch but occasionally at a different pitch. The patient silently counts the rare tones. An extra brain wave occurs at about 300 msec after each rare tone. This peak is referred to as the P300. The P300 is the equivalent of the brain saying, “Oh, that’s it! Count it!” That ERP peak latency is delayed in dementia but not in depression. ERPs can also be produced with visual stimulation.

The P300 is often delayed in MS patients (162). Earlier auditory cortical peaks were also delayed, so the degree of delay may represent a mixture of simple sensory system dysfunction plus delayed cognitive processing speed itself. The majority of MS patients studied showed delayed P300, worse among patients with secondary progressive MS (163). Visual P300 showed smaller amplitudes in the frontal regions of patients with frontal MRI lesions (164). During a serial ERP study, new P300 delays developed (165). Those P300 changes did not correspond to EDSS changes.

The role of P300 testing in MS patients remains to be clarified. It may help to establish and measure objectively one facet of cognitive impairment.

**MULTIMODALITY EP TESTING**

It is worthwhile to compare the three EP modalities against each other, and also compare multimodality EPs against MRI and cerebral spinal fluid findings in MS. This is useful for comparing which modality is most sensitive for clinical diagnostic purposes in MS overall. Such comparisons can also be helpful in planning strategies for research studies, such as therapeutic trials.

Chiappa (23) has summarized the aggregate results of 26 to 31 original research reports of the rate of abnormalities of the three sensory EP modalities (24–48,51,86–108,114–135). A summary of that is provided in Table 1. Overall, this set of data encompasses several thousand patients in several dozen research reports. Several specific features should be pointed out. The overall rates of abnormality are highest for pattern VEPs and lowest for BAEPs. SEPs have abnormality rates nearly as good as VEPs, even exceeding the latter’s rate in the possible and probable MS
category. Lower extremity SEPs, from peroneal and posterior tibial nerves, are often abnormal than median nerve SEPs. Silent lesions, i.e., EP abnormalities despite no signs or symptoms in the sensory modality, are seen in one-third of the patients tested overall.

Among patients with a more severe degree of MS, SEPs are even more likely to be abnormal. In one study (166) of all three EP modalities simultaneously in 101 patients with chronic progressive MS, pattern VEPs were abnormal in 75%; BAEPs in 48%; and median nerve SEPs in 93%. In most of these cases the EPs were abnormal but still present. This latter fact is important if one wishes to follow changes in EPs over time, since there is room in such cases for either improvement or deterioration. A more detailed comparison of the three sensory EPs modalities recorded in this study is presented in Table 3. Similar results have been reported in children with MS (167).

How useful are EPs in providing diagnostic information in patients being evaluated to rule out MS? Hume and Waxman (168) followed for two and half years, 222 patients suspected of having MS. During follow-up, 48/222 initially suspected of MS developed clinically definite MS. Among these 48 patients, 90% had had abnormal EPs during their initial clinical work-up. In 65% of these 48 patients, the EPs provided positive diagnostic evidence of a silent lesion previously unsuspected by the clinician or the patient. In the remaining 25% of these 48 patients, the EPs provided confirmatory information only. Among these same 48 patients, the VEPs were

### Table 3  Evoked Potentials Found Among 101 Patients with Chronic Progressive MS (Left and Right Sides Scored Separately)

<table>
<thead>
<tr>
<th>Pattern visual EPs</th>
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<tr>
<td>P100 latency (median)</td>
<td>119 msec (normal &lt; 105)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>50/202 (25%)</td>
<td></td>
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<tr>
<td>Present but delayed</td>
<td>132/202 (65%)</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>20/202 (10%)</td>
<td></td>
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<tr>
<td>Median P100 amplitude</td>
<td>4.0 μV</td>
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<tr>
<th>Brainstem auditory EPs</th>
<th></th>
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<tbody>
<tr>
<td>I–V interpeak latency (median)</td>
<td>4.4 msec (normal &lt; 4.6)</td>
<td></td>
</tr>
<tr>
<td>V/I amplitude ratio (median)</td>
<td>64% (normal &gt; 50%)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>105/202 (52%)</td>
<td></td>
</tr>
<tr>
<td>V present but abnormal</td>
<td>24/202 (12%)</td>
<td></td>
</tr>
<tr>
<td>V absent</td>
<td>63/202 (32%)</td>
<td></td>
</tr>
<tr>
<td>All five peaks absent</td>
<td>2/202 (1%)</td>
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<table>
<thead>
<tr>
<th>Median nerve somatosensory EPs</th>
<th></th>
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<tbody>
<tr>
<td>Normal</td>
<td>15/202 (7%)</td>
<td></td>
</tr>
<tr>
<td>N9 absent</td>
<td>0/202</td>
<td></td>
</tr>
<tr>
<td>N13 absent</td>
<td>70/202 (35%)</td>
<td></td>
</tr>
<tr>
<td>N20 absent</td>
<td>115/202 (57%)</td>
<td></td>
</tr>
<tr>
<td>N20 latency (median)</td>
<td>26 msec</td>
<td></td>
</tr>
<tr>
<td>N20 amplitude (median)</td>
<td>0.8 μV</td>
<td></td>
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</tbody>
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Small adjustments to normal limits for individual patients were made for age, gender, and height (details not shown here). Absent peaks were excluded from median latency determination here. Somatosensory normal limits were N20-N9 < 10.5 msec, N20-N13 < 7.0 msec plus N13-N9 < 4.3 msec; absolute latencies of N20 were not used when assessing normality.

**Abbreviations:** EPs, evoked potentials; MS, multiple sclerosis.

**Source:** From Ref. 166.
positive in 53%, somatosensory in 26%, and the brainstem auditory in 13%. The VEP was the only positive EP in 14 patients (30% of the patients who developed definite MS), somatosensory in 5 (11%), and brainstem auditory in none. In the same study, 18 of the original 222 patients eventually received a diagnosis other than MS. Among these patients, EPs were usually normal. Abnormal EPs were occasionally seen in other disorders, e.g., an abnormal VEP in a patient with vasculitis. Overall, the false positive rate for EPs appeared to be about 13% in this rule-out MS diagnostic paradigm.

Others have reported similar findings. MRI abnormality is more reliable for predicting a future diagnosis of MS compared to CSF or EPs, although the EPs did provide helpful information (57,169,170). EPs abnormalities are also seen in some other disorders, so they are not specific to MS (171).

Hume and Waxman (168) also assessed the likelihood of disease progression in patients initially evaluated for possible MS. They found a 71% chance of clinical deterioration over two and half years if the patient had abnormal EPs, whereas there was only a 16% chance of clinical deterioration over the same time span if the patient had normal EPs. Several CSF measures were not so accurate in predicting deterioration. Both VEP and MRI progression over time predict EDSS changes, and together they can provide a mathematical estimate of clinical progression (172).

MRI has been compared to EPs in several studies. Overall, multimodality EP testing is abnormal about as often as MRI among patients with definite or probable MS (56,62,66,109–113,168,173). Either type of test finds abnormalities in approximately 70% of patients evaluated across these various studies. Indeed, multi-modality EP testing found slightly more abnormalities than MRI in several reported series (62,66,111,113). BAEPs appear to be better than MRI for detecting lesions in the pons (109,112). VEPs also appear to be better than MRI at detecting optic nerve lesions in MS. Some research reports have evaluated how well each type of test finds multiple abnormalities, thereby helping to confirm the multifocal nature of the disorder under evaluation. By this criterion, MRI can show multiplicity of lesions more effectively than multimodal EPs (62,112). MRI and multimodal EPs were similarly effective in predicting an MS diagnosis, its course, or severity (65,168,173–175).

The likelihood of an MS diagnosis is enhanced by the use of EPs alone among 7/25 (28%) patients studied by Gilmore et al. (113). In the same study, brain MRI results alone made the diagnosis more likely in 4/25 (16%) patients. Among the remaining 14/25 (56%) patients, both the EPs and the MRI made the diagnosis more likely by providing evidence of additional lesions and abnormalities typical of demyelinating disease. In a larger patient group studied by the same authors, EPs found a second, silent lesion in 21/58 (36%) of patients, and brain MRI in 18/58 (31%) of patients.

In comparison to oligoclonal banding and similar CSF changes, multimodal EPs were slightly more likely to be abnormal in early or possible MS (65,133,168, 176–180), although specific results did vary among reports. When they are abnormal, EPs predict, with a higher degree of uncertainty, that the patient will deteriorate after a several year period. Patients with normal results on both EP and CSF studies are most likely to remain stable during follow-up. There is no further relationship between CSF changes and any particular type of EP abnormality.

In a formal technology assessment, the American Academy of Neurology (AAN) Quality Standards Subcommittee looked at EPs in MS (181). They used a structured literature review to assess the usefulness of EPs in identifying clinically silent lesions in patients with suspected MS. On the basis of this review and analysis,
the AAN formally recommends using VEPs and SEPs to search for clinically silent lesions. For BAEPs, there is a trend in the direction of usefulness to search for silent lesions, but the magnitude of an effect is much less than for VEPs and SEPs. The report notes that there are other reasons for using EPs in MS beyond just searching for silent lesions. These may include: to aid in localizing lesions, to confirm clinically ambiguous lesions or the organic basis of symptoms, and to suggest demyelination as the cause of a suspicious lesion.

Finally, it is appropriate to look at the comparative resource utilization of EPs and MRI. In the United States, the medicare fee schedule allows 29.12 relative value units (RVUs) for brain MRI, and 29.42 each for cervical and thoracic MRI. In contrast, all four EP tests together are valued at 8.66 RVUs. This includes 1.24 RVUs for VEP, 3.31 RVUs for your extremity SEP, and 4.11 RVUs for BAEP. In this relative value assessment of resource utilization, all four sensory EP tests cost 30% as much as one brain MRI; or, 10% of the cost of combined brain, cervical, and thoracic MRI.

Overall, most investigators have concluded that the two types of tests are complementary to each other, one assessing anatomy and the other assessing physiology. Each has its own niche in the diagnostic evaluation paradigm.

USE OF EPs IN MS THERAPEUTIC TRIALS

EPs are clearly a useful measurement for MS therapeutic trials (1). Testing can be repeated annually or semi-annually. The costs associated with such testing are reasonable, and can be integrated into most therapeutic trial budgets. Visual testing seems to be the best modality for therapeutic trials because of its ease of measurement. Grouped data provide a reliable way to track accumulating disease burden (44,166,182).

In one large trial of azathioprine and steroids, both visual and median nerve middle-latency sensory EPs proved to have approximately equal statistical significance. These two modalities were superior to BAEPs in predicting the overall outcome of the study (166). VEPs have the advantage that they do not require the annoying somatosensory electrical shocks on the wrist. The SEPs take more than twice as long to perform compared to VEPs. The somatosensory short-latency EPs, using peaks between 13 and 22 msec, are often unsatisfactory for therapeutic trials because those peaks tend to be absent in a large portion of patients who would be entered in such a trial. The middle-latency somatosensory peaks are preserved in essentially all MS patients (166), but most laboratories have much less familiarity than those with short-latency SEPs.

The use of EPs in therapeutic trials is commensurate with the recommendations of the Ad Hoc Working Group on the design of clinical studies to assess therapeutic efficacy in MS (183). In the report it was stated that, “the unpredictability of the clinical course of MS makes it necessary for the investigator to be particularly critical in choosing methods for assessing the changes in patients relative to any putative therapy ... the frequent occurrence of lesions in clinically silent areas provides part of the impetus for seeking to include laboratory parameters in modern therapeutic trials ... made determinations that seem to be potentially most useful at the time of writing include visual evoked response (and several immunological tests).” This belief has been substantiated in at least one well-designed thorough study of EPs in a therapeutic trial. The use of magnetic resonance scans now also
appears to be a highly recommended testing modality for following patients through therapeutic trials. The quantifiable aspects of MRI testing include the amount of plaque load, measure in cubic millimeters, and the number of plaques seen. Quantifying these require considerable sophistication, cost, time, and effort. The sensitivity of MRI seems superior to EPs for this purpose because the MRIs cover a much greater volume of deep white matter. The biggest disadvantages to the MRI study lie in the much greater amount of money, effort, and expertise needed to use the technique correctly in MS therapeutic trials. In many trials, the VEPs may end up yielding the same general outcome data in a sensitive, objective, and reproducible way. In the design of a therapeutic study, it is therefore important to weigh the advantages and disadvantages and the cost effectiveness of the testing approach to be taken. In this author’s opinion, VEPs will be found to be the more cost-effective alternative of the two approaches in many individual trials.

Some skepticism has been voiced about the usefulness of EPs in monitoring the course of MS disease activity. This is in part because the EPs often remain quite abnormal even when the MS becomes relatively inactive (51). This is actually, however, an advantage of EPs since they tend to worsen progressively in untreated MS. EPs can detect the physiologic remnants of a new plaque that appeared long before and may gradually have become relatively inapparent on MRIs. The gradual progression of EPs provides an objective, quantified measure that parallels progression in EDSS (136,174).

EPs are not redundant with any signs or symptoms that can be easily determined by physical examination or detailed history. This is because the EPs tend to pick up many silent lesions that are not reflected in any particular way in the physical examination or history.

The design of EPs for therapeutic trial is also important. There are appropriate ways to carry out the study, and other ways in which the testing may be of little or no benefit. This is particularly important when it comes to the scoring of the tests. The VEPs ought to be scored in terms of the actual latency values of the EPs. Test–retest differences ought to be reassessed by direct, careful comparison of the actual EP traces rather than by completely separate scoring of the individual traces. In this way, the reader can make sure that the scoring is based on exactly the same portion of the EP peaks when separate repetitions are scored. This substantially reduced the trial-to-trial variability. The statistics done with EPs also ought to be done using the actual latency values. Year to year comparisons in the therapeutic trial can then be carried out using parametric statistics. This is far superior to the better/worse/unchanged, or changed/unchanged or normal/abnormal scoring that has been used in unfortunately large number of reports of EPs in therapeutic MS trials. The latter techniques are not statistically powerful and defeat the goals of using a quantified tool in this type of scientific study.

EPs have shown to be of value in several therapeutic trials. One is the trial of azathioprine, antilymphocite globulin, and steroid trial reported by Mertin et al. (184). In that study, visual, brainstem auditory and short-latency median nerve SEPs were performed at the beginning and end of the 15-month treatment course. EP changes were scored as better, worse, or unchanged. The authors found that the auditory and short-latency somatosensory EP tests were difficult to interpret because of the complexity of the multiple peaks and absent peaks. The simpler, visual pattern reversal EPs deteriorated in their control group, whereas the VEPs were more stable \( (P = 0.06) \) in their immunosuppressed group. The authors also found a small clinical improvement in the treatment group, compared to the controlled group, although
this was not statistically significant. One can learn from this study that the VEPs were better at detecting changes than the other modalities and that the VEPs were able to show a therapeutic effect, even when the clinical data otherwise showed a trend that did not reach a statistical significance.

In a serial study of EPs in 19 relapsing–remitting MS patients over six months of steroid treatment, a positive correlation was found between EPs and clinical disability scores (185). Among 15 relapsing–remitting MS patients treated with interferon, VEP latencies showed mild improvement over two years. The authors concluded that VEP is a reliable index to follow progress at MS during therapy (186). Similar improvements were seen among 35 patients treated with steroid and cyclophosphamide and followed with MEPs (187). The MEP improvement paralleled the EDSS. P300 improvement was seen during short-term steroid therapy (188) and for MEP (189).

In the UCLA study of azathioprine with or without steroids in a three-year, double-blind, placebo-controlled therapeutic trial in chronic progressive MS (166), EPs substantially outperformed routine physical examination and disability scales in predicting the study outcome. Visual, brainstem auditory, and median nerve middle latency SEPs were performed annually during this three-year study. Treatment-related visual and somatosensory EP changes became statistically significantly different in one year before corresponding differences were seen in the Standard Neurological Examination scores. Data regarding gradual changes is shown in Figure 4. Note that at the bottom of each graph, the statistical significance of the results is shown. For the VEPs, the probability of a treatment related difference was $P = 0.13$ even at year one in this three-year study. By year two, the difference had grown to $P = 0.02$, considered statistically significant since it is $P < 0.05$. By year three, the statistical significance of the difference had grown to $P = 0.002$, with the double drug treated group seeming to be stable over the course of this therapeutic trial. The statistical significance of this VEP difference was substantially greater in degree that was true for the standard neurological examination score, which only reached a $P = 0.04$ level of statistical significance by the third year of this study. Overall, the authors in that study concluded that the statistical significance of EP changes was substantially greater than that seen for other clinical scales. The degree of significance was increased by using EP latency values, rather than simple criteria for change. EPs were considered to be a sensitive, objective measurement useful in MS therapeutic trials.

In comparison, in that same study, EPs were evaluated using the much more common better/worse/unchanged criteria. When the VEPs were analyzed using a 10, 7, or 5 msec criteria for “change,” the group differences using a chi square analysis did not reach statistical significance. This should help to drive home the very important lesson that EPs in a therapeutic trial must be applied by taking advantage of the quantified nature of actual latency values, rather than the statistically much less powerful and much less effective better/worse/unchanged, or normal/abnormal or changed/unchanged types of qualitative schemes for scoring.

Other studies have also looked at EPs during MS therapeutic trials. Most of those investigators have not found EPs to be helpful. Some of these studies failed to use EPs in the quantified manner described above. In some other studies the treatment used was not effective, and the EPs were therefore quite correct in saying that there was no effect. For example, hyperbaric oxygen tests in MS did not change EPs (190,191). Acyclovir produced no clinical or EP effect (192). High dose methylprednisolone was found to cause no change in any three EP modalities when retested at
one week or at one month despite some clinical improvement in some patients (193). Among the several steroid regimens compared by LaMantia et al. (194), EP changes paralleled clinical changes at six months. In natural alpha interferon trials, EPs have confirmed the clinical findings using the disability status scale and the scripps neurological rating scale (195). Studies of plasmapheresis found that EPs tend to corroborate clinical changes found in five reports (117,134,196–198).

Figure 4 Effects of azathioprine and steroids on evoked potentials and on a standard neurological examination score, during a three-year study in 57 patients. The three drug treatment groups are shown (AP = azathioprine, AM = azathioprine plus steroids, PP = placebo only). In each case increasing scores represent worsening. Statistical significance is shown above each horizontal axis. These data show that the AM group remained stable or even had slightly improved scores for each type of measurement. Error bars represent the standard error of the mean. Overall, the statistical significance of the group differences can be seen earlier and more strongly in the evoked potential data. Source: From Ref. 166.
Overall, EPs seem to be a reasonable cost-effective tool, that can be used to great advantage in clarifying and adding statistical significance to the results of an MS therapeutic trial. They are especially useful when used in a quantified manner.

REFERENCES


268 Nuwer
Managing the Symptoms of Multiple Sclerosis

Randall T. Schapiro
The Schapiro Center for Multiple Sclerosis, Minneapolis Clinic of Neurology, and University of Minnesota, Minneapolis, Minnesota, U.S.A.

INTRODUCTION

The management of multiple sclerosis (MS) in the current millennium clearly has emphasized stabilization of the disease itself. The past decade has seen the common use of disease modifying therapies and the future is bright for more treatments that can alter the course of MS. However, from a very practical point of view, the management of the symptoms caused by the destructive process of MS remains of major importance. Symptom management can improve the quality of life so significantly that it can make the difference in a person being able to live in today’s society or not. There are many symptoms that occur regularly in MS. There are ways to manage those symptoms. This chapter discusses the medical management of MS symptomatology. Chapter 11 discusses in depth the rehabilitation of MS. It is essential to realize that managing symptoms and rehabilitation cannot stand alone. The proper management of MS symptoms involves medication and rehabilitation done simultaneously on an ongoing basis. When these occur together, symptom management in MS becomes real and alive!

FATIGUE

The single most common and disabling symptom in MS is fatigue (1). Five different fatigues can be found contributing to the alarmed feeling that bothers most with MS. Normal fatigue occurs in those with the disease just as it does in those without MS. This is especially the case if the person is trying to prove competence by doing more than expected. The management strategy is to understand the situation and recognize that fatigue of this sort is not damaging but simply tiring. People who have MS are not fragile, and while the idea is not to test the system to see how far one can go before permanent problems occur, one can go pretty far and still live to tell the tale. Occupational therapists teach energy conservation and effective ways to treat
activities of daily living. They can be helpful in exploring new and efficient ways to do normal tasks with less fatigue.

MS can lead to depression and depression can lead to fatigue. This is especially important to understand because there are treatments for depression that are efficient and effective. Demyelination in the brain typically leads to changes in the neurochemistry in the brain (2). This may manifest as the signs of depression, with sleep disturbances, eating disturbances, and fatigue. The specific serotonin release inhibiting medications, of which there are many, can be of significant value because they not only treat depression, but also can energize in the process (3). Thus, these medications should accompany counseling for this specific type of fatigue.

Neuromuscular fatigue follows the repetitive stimulation of a demyelinated nerve. This “short-circuiting” type of fatigue presents as muscle fatigue with ongoing use of a muscle. It is best treated by rest, allowing the nerve and muscle to recover function. This is the reason that progressive resistive exercise must be done with caution, allowing time for the nerve–muscle combination to recover between repetitions.

Lassitude or MS specific fatigue is the term reserved for the overwhelming tiredness that comes with autoimmune diseases. This occurs with MS and is a sleepiness that is prevalent despite the absence of activity and after a good night’s sleep. Neurochemicals such as amantidine and fluoxetine are of benefit (3). Stimulants such as pemoline may be helpful but have been associated with liver disease making its use impractical (4). Other stimulants may be habit forming and are difficult to control. Modafinil has risen to become a popular agent for managing lassitude (5). It appears to have no specific dependence associated with its stimulation. Care must be taken not to provoke agitation with the combinations of medicines to help treat fatigue. Also care must be taken to prevent over sedation with the medications used to treat other symptoms seen in MS. Iatrogenic fatigue may be a necessary, but not welcomed side effect of aggressive management. Sleep disturbances need to be guarded against as they can be insidious and very fatiguing if not managed properly.

**SPASTICITY**

Spasticity is the result of an upper motor neuron dysfunction in regulation of impulses and neurochemistry. The presence of spasticity is not necessarily negative, as it may be present without significant weakness and it may be helpful in transferring techniques. However, if it is causing discomfort, aggressive treatment is not only appropriate but also necessary. Ambulation problems are the result of many different factors. These include spasticity, weakness, ataxia, sensory disturbances, and cognitive disturbances. Most of these are treated with rehabilitative techniques.

Removal of noxious stimuli is the first line of spasticity management. Pain anywhere in the body will exacerbate spasticity. That is true even if the pain is remote to the spastic extremity. Exercise follows removal of noxious stimuli in the management scheme. Following those two points comes the addition of medication. Baclofen (Lioresal) is usually the first medication utilized. It is effective at low to high dosing and the exact dose is determined by the response. Usually it is begun at 5 mg three times a day, but doses of 40 mg four times a day may be necessary for some. The side effects of weakness, sedation, and cognitive problems are the limiting factors. If baclofen is found to be ineffective on its own, tizanidine (Zanaflex) may be added to the baclofen for synergistic potential (6). These medications act with different mechanisms and thus may be additive. High
doses of baclofen may lead to weakness while high doses of tizanidine tend to pro-
mote fatigue and dry mouth. Because the side-effect profiles are somewhat different,
the choice of agent may be based on the specific situation (7). Thus, tizanidine may
be the primary agent if weakness is a prevalent symptom. Doses of 2 mg each day to
36 mg in divided doses may be necessary.

Spasms are common in MS and often occur during the night or just before
sleep. While baclofen and tizanidine are helpful for spasms, both clonazapam (Klo-
nopin) and diazepam (Valium) are also appropriate. That takes advantage of their
antispasticity and sedating potential. Clonodine is an anti-hypertensive agent, which
is a relative of tizanidine. It may be administered via a skin patch and can control
spasms if the blood pressure lowering effect is not too much for the individual.
Gabapentin (Neurontin), topiramate (Topamax), and other anti-convulsant medica-
tions may also be helpful in spasm management (8,9). Dopaminergic agents can help
spasms at fairly low doses and the serotinergic antagonist cyproheptadine (Periactin)
may treat spasticity with a high level of sedation (10,11).

Dantrolene (Dantrium) is often too weakening for many with MS, but it may
be helpful at low doses for the spinal form of the disease.

Botulinum toxin (Botox) can be helpful for significant single muscle spasticity
and/or spasms (12). This is especially true for small muscles such as seen in the hand
or face. Unfortunately, most spasticity and spasms seen in MS are in large muscles or
whole limbs and the treatment requires too much toxin to be practical. Motor point
blocks with phenol and surgical neurectomy are done less today.

There has arisen a fair amount of controversy regarding the use of canniboids
as a spasticity treatment (13). The legal issues surrounding canniboid use are such
as to make it not practical for individual trials. There are clinicians who believe that
the relaxing qualities of canniboid administration will relieve spasticity, but damage
to the lungs and the addictive potential clearly point to a cautious approach to
their use.

The baclofen pump has been phenomenal for intractable spasticity not mana-
ged by other approaches (14). The synchromed programmable pump has allowed
for relief of severe spasticity with minimal side effects. However, it involves a
surgical procedure and poses the problems of any mechanical device with a catheter
and occasionally the pump itself. It requires an experienced physician to implant and
control the dosage of the medication (usually different physicians).

WEAKNESS

The weakness seen in MS is usually due to decreased central conduction secondary
to demyelination. Occasionally deconditioning causes weakness. When that is the
case, a strengthening program will potentially bring the muscle back to normality.
However, there is usually a degree of decreased conduction, and progressive resistive
exercises lead to fatigue, as described above.

The old adage “use it or lose it” does apply to weakness in MS. Thus, even if
the muscle is neurologically weakened, it should be stimulated to prevent atrophy.
Thus, the therapist must carefully ferret out the muscles that can and should be
strengthened by exercise. Medication can boost nerve conduction. The aminopyri-
dines are potassium channel blockers, which allow for faster and more efficient
transmission in demyelinated nerves (15,16). They are chemicals and are available
in compounding pharmacies. However, the quality control is not universal and
the incidence of seizures is unacceptable. Studies continue to develop an FDA approved compound, which would, theoretically, lower the side-effect profile. Until that is approved, aminopyridine use is not recommended. Nonetheless, several reports indicate increased strength and endurance and decreased fatigue with aminopyridine use.

**URINARY DYSFUNCTION**

Urinary discomfort in MS is very common. The “MS bladder” can be big and boggy or small and muscular. In both cases the symptoms may be similar, including urgency, frequency, hesitancy, and incontinence. In the big bladder that fails to empty, the symptoms are secondary to overflow incontinence. In the small “failure-to-store” bladder, they are due to the hyperactivity of the bladder muscle. Diagnosis is the key to treatment. Residual urine determination can be obtained via ultrasound or catheterization. If the residuals are high, catheterization techniques may be essential. Stimulants such as urecholine are occasionally helpful and worth a try in selected individuals. Ataxia, numbness, weakness, and cognitive problems often make self-catheterization less desirable despite the appropriateness of the bladder for that technique.

Anticholinergic medication (oxybutynin, tolterodine, propantheline) can be titrated to slow the hyperactive bladder (17). Care must be taken with these, especially in the summer, as they can decrease the sweating response and inadvertently lead to hyperthermia and severe weakness.

Dyssynergia of the bladder and bladder sphincter is also common in MS (18). Urodynamic studies may be necessary to make this diagnosis. Alpha-blocking agents (terazosin, phenoxybenzamine) may aid in better emptying if this condition is present.

Nocturia is often a problem in MS. The constant ups and downs to remain continent during the night may contribute to significant fatigue the next day. DDAVP (desmopressin, anti-diuretic hormone) may slow down urine production enough to allow for more restful sleep (19).

**BOWEL DYSFUNCTION**

Bowel problems in MS are reasonably frequent, although not as typical as bladder dysfunction. The most common problem is constipation. Often this is due to self-imposed dehydration to control bladder frequency. Lack of physical activity also contributes to the problem. Both of these can be solved if the right attitude is instilled and the bladder is regulated without dehydration.

However, often a bowel program is necessary. This begins by understanding that the best time for a bowel movement is after a meal. This takes advantage of the gastrocolic reflex. The addition of a bulk-forming substance (e.g., Metamucil, Fibercon, Citrocel) can be important. If that fails the addition of a gentle mechanical stimulant such as a glycerine suppository on the third day of constipation often works. The idea is to have a bowel movement about every three days or less. If that fails stimulants in the form of Dulcolax suppositories may be necessary. Stimulation from above with milk of magnesia or polyethylene glycol (Miralax) or lactulose is sometimes necessary to treat especially refractory constipation. Stool softeners may also be added.
Sometimes the problem is the opposite, with urgency and incontinence. The goal in this situation is to bulk up the stool to allow more time for the bowel movement sensation. Often transferring and undressing techniques become important here. The use of Metamucil as a bowel regulator is the most frequent management suggestion. In this situation, more Metamucil with less fluid is utilized in order to allow the Metamucil to absorb excess bowel fluid, making the stool firmer. Bowel movements on a schedule in the morning allow for more freedom during the day.

**SEXUAL DYSFUNCTION**

The management of sexual dysfunction continues to evolve. There was a time when little could be done for the man or woman with MS who had sexual difficulties. For the man with erectile dysfunction, treatment in the 1980s meant a penile prosthesis. While these evolved into very functional and useful pieces of equipment, their use in MS continues to diminish because of the advances that are not surgical in nature. Prostaglandin (aprostadiol) can be injected intracorporally giving a very firm and usable erection (20). Vacuum tubes that draw blood into the penis were popular for a short time. Their clumsiness and perceived lack of efficacy made them less popular. The aprostadil could be given via the urethra in the form of a suppository (MUSE) (11). The most popular treatments have become oral agents which can give an erection with relatively minimal side effects. There are now three available for use; sildenafil citrate, vardenafil HCI, and tadalafil can be taken around a half an hour before sexual activity and will allow a very natural, usable erection in many men with MS who previously could not achieve one (21). They require love making to work and have various half-lives making spontaneity more realistic.

Women who have decreased lubrication have their choice of very natural vaginal lubricants. Vibrators can produce stimulation in numb areas and the application of cold (a frozen bag of peas) can decrease pain and burning and give stimulation. An FDA approved device, the EROS device, allows for gentle vibration with suction of blood into the clitoris. It allows for the re-introduction of stimulation that can be self and partner directed (11,22).

The key to beginning the management of sexual dysfunction is to ask about it. Too often the topic is avoided and thus the problem is not treated. Insurance companies have often chosen to see this as a condition that does not require treatment. This goes against the majority opinion among people who want to be sexually active but cannot be as today and as they were in the past. Today the methodology to make this possible exists.

**PAIN**

Pain is very common in MS (23). Over half of those with MS will have pain of some sort (24). In some of these patients, the problem is quite obvious, with pain due to orthopedic, joint or back problems that may have occurred because of gait deviations, altering the normal joint relationships. These need orthopedic intervention with correction of the problem.

However, often the pain is a burning, irritative pain, a dysesthesia. The neuropathic pain may occur anywhere in the body. It is likely due to demyelination within the sensory tracts in the brain and spinal cord. Some antiseizure medications have
been helpful in controlling the pain of MS (11). Carbamazepine has been used for many years for the nerve pain of neuralgia, especially trigeminal neuralgia that is seen reasonably frequently in MS. Doses of 800 mg or more are sometimes required, and this often leads to significant fatigue. Gabapentin has been helpful for neuropathic pain without the severe fatigue seen with carbamazepine. Doses of gabapentin must often reach 2400–3600 mg for an optimal effect. Other similar medications: topiramate, lamotrigine, tiagabine, levetiracetam, and oxcarbazepine have a similar effect. Occasionally other neurochemical agents, e.g., misoprostol may serve as adjuvant medication (25,26).

The tricyclic antidepressants, including amitriptyline, are utilized, but again, they are quite sedating. They may allow for help in sleeping in the case of pain.

TREMOR

Tremor is one of the more disabling symptoms. It is also among the most difficult to treat. It is not unusual to see tremor in a person who otherwise has retained good strength. It is also not unusual to see it in the more cognitively impaired, giving meaning to the descriptive term *cerebellar-cerebral* MS. The tremor is typically of the action variety. No real help is afforded by exercise; thus the medical management is particularly important. There are not drugs that work universally in tremor management but many have the potential to help sometimes. None of these pharmaceuticals were introduced specifically for tremor management and are thus all used “off-label.” Most have been poorly studied. Nonetheless, by experience, a variety has proved to be helpful.

Propranolol (Inderal) is a beta-blocker that clearly helps with essential tremor and is often of some help with the cerebellar tremor of MS. Doses of over 160 mg are often necessary to get an effect (27). Primidone (Mysoline) will occasionally tone down the tremor with less than anticonvulsant dosages of 150 to 300 mg per day (28). Clonazapam (Klonopin) will provide a calming effect, which can diminish the tremor as well its relative, diazepam (Valium). The tuberculosis medication, isoniazid (INH) in high dosage (300 mg three times per day) will, in some, decrease the gross tremor often described as “rubro.” Liver and blood toxicity must be guarded against (29). Ondansetron (Zofran) in dosages of 8 mg three times a day has had a better effect than most medications but its cost is especially prohibitive (30). Buspirone (Buspar) in dosages of 10 to 15 mg four times a day will occasionally help diminish tremor (31).

Often various combinations of these medications are necessary to get an effect, and trial and error is the rule.

Surgical procedures involving lesioning the extrapyramidal system proved more dangerous than helpful in the late 1960s. Now the question remains as to whether electrical stimulation of these areas as done in Parkinson’s disease would be helpful for the tremor of MS. There have been no significantly large studies to give an indication of its value in this situation.

VISUAL DYSFUNCTION

Visual problems in MS are very common. Decreased vision due to disease of the optic nerve or tracts is particularly frequent due to their highly concentrated myelination.
High dose IV corticosteroids (methylprednisolone, 1000 mg per day for 3–5 days) often will shorten the course of acute visual loss secondary to optic nerve inflammation (32). There are no good data to indicate that a low-dose steroid (usually given orally) makes a difference. Some have interpreted the study data as showing a negative effect with oral treatment, but that interpretation was made without a study designed to look at this specific question and must therefore be held in doubt.

Diplopia secondary to internuclear ophthalmoplegia or isolated brainstem involvement of the extraocular muscles and nerves is annoying. Steroids can speed recovery in this situation as well, but often the healing is slow and incomplete. The brain is usually capable of fusing the images even in the face of muscle imbalance if patching of an eye is not too aggressively done early in the course of the problem.

PAROXYSMAL SPASMS

A unique symptom that occasionally occurs in MS is that of paroxysmal electrical short-circuiting in the spinal cord. This results in repeated episodes of spasm or sensory disturbance. These are called tonic spasms and can be frightening, especially if misunderstood. The spasm usually begins in the upper extremity but may spread to the legs or even the face. It lasts for seconds and may recur very frequently and then settle down without rhyme or reason. It is treated with anticonvulsants, particularly carbamazepine. Fairly low doses usually control the problem and, after it is settled, the medication can usually be withdrawn.

PATHOLOGICAL LAUGHING/CRYING

Pathological laughing and crying is another symptom linked to diffuse brain damage. While it may occur with small strokes, it is not unusual with the more cortically involved MS pathology. It is a symptom of pseudobulbar palsy. The individual cries or less commonly laughs inappropriately and uncontrollably. Tricyclic antidepressants (amitriptyline) have been helpful in gaining control of this embarrassing symptom.

DEPRESSION

Depression is a primary symptom seen in MS. It appears secondary to the neurochemical alterations that occur from the organic changes within the brain. As such it needs to be watched for in all with MS and treatment with antidepressant medications should not be feared or avoided. The newer antidepressants offer depressant management without the sedation of the treatments of decades ago.

CONCLUSION

The management of MS has changed in the past 10 years. Nonetheless, the backbone to managing MS properly remains the symptom management of the disease. Today, we can truly begin to manage the disease itself, the symptoms of the disease, and the person with the disease. This truly improves the quality of life for those with MS.
REFERENCES


Rehabilitation: Its Role in Multiple Sclerosis

George H. Kraft
Department of Rehabilitation Medicine and Neurology, University of Washington, Seattle, Washington, U.S.A.

Anjali N. Shah
Multiple Sclerosis Program, University of Texas Southwestern Medical Center, Dallas, Texas, U.S.A.

INTRODUCTION

When a multiple sclerosis (MS) patient walks into a specialist’s office, they do not say, “Doctor, please help me. My T-cells are attacking my myelin.” Rather, they are more likely to ask for help with a foot-drop, weakness, memory or bladder problems, pain, or state that things are not going well at work. Thus, at the outset, MS patients ask for help with their functional impairments or disabilities. They are asking for rehabilitative services (1). Rehabilitation is still the only way to improve function in MS (2). A patient can be improved from bed-bound status by giving her a wheelchair [from an Expanded Disability Status Scale (EDSS) of 8.0 to 7.0] and from wheelchair reliant (we must stop using the pejorative term “wheelchair bound”) to ambulatory with an orthosis or walker (from 7.0 to 6.5).

MS is uniquely difficult to rehabilitate, as patients with this disorder may display concurrent weakness, spasticity, sensory loss, ataxia, dysmetria, tremor, pain, cognitive impairment, depression, and fatigue—a combination of problems seen in no other disorder. Furthermore, the disease can worsen over time and have an unpredictable course, requiring a periodic reassessment of rehabilitative treatments. Finally, an exacerbation can occur at any time.

The importance of providing these services is emphasized in one of the several position papers published by the National Multiple Sclerosis Society (NMSS). The Expert Opinion Paper, endorsed by the Medical Advisory Board of the NMSS, states “Rehabilitation in MS is a process that helps a person achieve and maintain maximal physical, psychological, social, and vocational potential, and quality of life (QOL) consistent with physiologic impairment, environment, and life goals. Achievement and maintenance of optimal function are essential in a progressive disease such as MS,” and “The physician should consider referral of individuals with MS for
assessment by rehabilitation professionals when there is an abrupt or gradual worsening of function or increase in impairment that has a significant impact on the individual’s mobility, safety, independence, and/or QOL (73).”

An important point, which needs to be stressed here, is that an MS patient, whose disease gets worse over time, needs a periodic reappraisal of services needed. For example, a moderate level of spasmolytic medication may not be adequate if the patient’s spasticity worsens. Consequently, these periodic reevaluations need to assess all aspects of a patient’s deteriorating function; the full spectrum of symptoms needs to be assessed and individualized treatments should be modified. Consequently, return visits may be lengthy. In our experience, such meticulous reassessment and readjustment of interventions is, unfortunately, rare in the practice community.

Following are some of the common symptoms of persons with MS and their rehabilitative management strategies.

**FATIGUE**

Some years ago, we carried out a comprehensive survey of patients’ needs, asking about the problems they were having and services needed (Table 1) (3). In our experience, those needs have not appreciably changed, although research has improved our understanding of causes and management strategies.

What needs do patients have? It appears that the most common problem about which patients complain—which was not reported before 1984 (4)—is fatigue. More recent work suggests fatigue affects 75% to 90% of MS patients (5–10). The QOL is significantly worse in patients with fatigue. Fatigue can greatly hinder and impede a person’s ability to perform activities of daily living (ADL) or be employable. MS patients experience two types of fatigue (i) fatigue as a result of exertion or (ii) lassitude regardless of activity level (10).

<table>
<thead>
<tr>
<th>Symptom present</th>
<th>No ADL difficulty</th>
<th>With ADL difficulty</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>21</td>
<td>56</td>
<td>77</td>
</tr>
<tr>
<td>Balance problems</td>
<td>24</td>
<td>50</td>
<td>74</td>
</tr>
<tr>
<td>Weakness or paralysis</td>
<td>18</td>
<td>45</td>
<td>63</td>
</tr>
<tr>
<td>Numbness, tingling, or other sensory disturbance</td>
<td>39</td>
<td>24</td>
<td>63</td>
</tr>
<tr>
<td>Bladder problems</td>
<td>25</td>
<td>34</td>
<td>59</td>
</tr>
<tr>
<td>Increased muscle tension (spasticity)</td>
<td>23</td>
<td>26</td>
<td>49</td>
</tr>
<tr>
<td>Bowel problems</td>
<td>19</td>
<td>20</td>
<td>39</td>
</tr>
<tr>
<td>Difficulty remembering</td>
<td>21</td>
<td>16</td>
<td>37</td>
</tr>
<tr>
<td>Depression</td>
<td>18</td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td>Pain</td>
<td>15</td>
<td>21</td>
<td>36</td>
</tr>
<tr>
<td>Laugh or cry easily (emotional lability)</td>
<td>24</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>Double or blurred vision, partial or complete blindness</td>
<td>14</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td>Shaking (tremor)</td>
<td>14</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>Speech and/or communication difficulties</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Difficulty solving problems</td>
<td>12</td>
<td>9</td>
<td>21</td>
</tr>
</tbody>
</table>

*Abbreviation: ADL, activities of daily living.*
The first type represents peripheral fatigue. This is usually due to muscular fatigue secondary to muscles weakened by MS. The second type, central fatigue, is perceived at the central nervous system (CNS) level and often a subjective assessment. Patients with central fatigue often complain of a constant feeling of tiredness. The mechanism of central fatigue is not fully known; this makes treatment for it difficult. It appears to be a heterogeneous disorder that may involve the pyramidal tract, sleep, anxiety, depression, immunoactivation, and perhaps the mechanism of brain plasticity (9). When treating patients for fatigue, it is important to acknowledge contribution from other factors (comorbidities, depression, stress, insomnia) and to review a patient’s medication list for drugs whose side effects include fatigue (e.g., tizanidine or orbaclofen). Nonpharmacologic interventions such as psychotherapy and exercise can improve QOL and appear to decrease fatigue. From a functional point of view, it is essential to evaluate for spasticity. Increased tone in a limb—especially those involved with gait—can lead to an increased energy expenditure, thus increasing the amount of fatigue.

The following interventions have been identified as treatment for fatigue in MS.

**Behavioral Therapy**

Lack of control of the environment (environmental mastery) is one of the best psychosocial predictors of global fatigue and fatigue-related distress for MS patients (11). Techniques to enable the patient to learn to control the environment may help fatigue (12).

**Medications**

Amantadine (Symmetrel®), modafinil (Provigil®), and pemoline (Cylert®) are the most common medications prescribed to treat fatigue in MS patients.

Amantadine (Symmetrel) is an anti-viral agent with dopaminergic qualities. Its mechanism of action in treating fatigue is unknown. Dosage is 100 mg twice a day; it appears that this is the optimal dose for almost all amantadine-responsive patients (perhaps 2/3 of MS patients with fatigue). The most common side effects include ankle edema, nervousness, sleep disturbances, and livedo reticularis, although it is tolerably benign in most patients. Several short-term, randomized controlled trials (RCTs) have shown a modest benefit of amantadine over placebo in measures such as ADL, QOL, and fatigue (13–16).

Modafinil (Provigil) is a novel “wake promoting agent,” and is used to treat excessive daytime sleepiness as well as narcolepsy. A phase II clinical trial evaluating modafinil at 200 and 400 mg versus placebo found that modafinil 200 mg/day given every morning for two weeks in MS patients with expanded disability status scale (EDSS) scores ≤6.0 significantly improved fatigue scores on the Fatigue Severity Scale, Modified Fatigue Impact Scale, and Visual Analog Scale compared to placebo (8). The side-effect profile was relatively mild and included headache, nausea, anxiety, and dry mouth. Aesthenia (worsening fatigue) was experienced more often in patients taking 400 mg/day of modafinil compared to the 200 mg/day dosage. Finally, patients who continued on the 200 mg daily dosage (unknown total number of patients) reported that they did not develop a tolerance to the drug. Caution should be given that the medication has a 15+ hour half life and should be given only in the morning.

Although we have traditionally managed fatigue with a variety of medications, recent research suggests that it is possible that we may be doing to a disfavor some of
these patients. Although many MS patients are disabled by their fatigue and need to aggressively treat it with medications, the sense of “tiredness” noted by some patients may be related to the progressive establishment of new brain traces “plasticity” occurring in a condition which produces ongoing neural degeneration. Clinicians often note a disconnection between the marked brain atrophy present in an individual patient (implying significant loss of pre-formed neural pathways) and the ability of such a patient to function. To function as well as they do, extensive plasticity—confirmed by imaging studies—has occurred in these patients (17).

What is required for plasticity to occur most efficiently? Studies in rodents demonstrate that new learning requires fairly immediate slow-wave deep sleep to encode newly learned information (18–20). Additionally, studies in volunteers confirm this in humans (21). It appears that the consolidation of new brain traces for efficient learning requires a fairly immediate period of slow-wave sleep to allow for the “offline” processing required for new synaptic plasticity (22). Consequently, the question arises as to whether it would be better for MS patients to have more periods of deep sleep rather than take drugs to stay awake. Are tiredness and fatigue trying send to the message “Give this brain sleep?”

Energy Conservation Techniques

Many MS patients learn how to use compensatory techniques to manage their fatigue. These include learning to recognize personal limits, scheduling activities around times when they are at peak energy level, taking naps, and using assistive devices to ambulate. A modification of a six-week community-based energy conservation course developed by Packer et al. was studied in MS patients. Seventy-nine patients enrolled and the study was divided into a six-week control group, and a six-week intervention group. The study concluded that the energy conservation program reduced the impact of fatigue, increased self-efficacy, and improved QOL. Carryover of the positive effects was also achieved (23).

Cooling Vest

In heat-sensitive MS (HSMS) patients, fatigue is often improved by utilizing this modality. This will be covered in the section entitled “Body Cooling.”

WEAKNESS AND SPASTICITY

These two symptoms should be treated together as it is not uncommon for a patient to appear to be weak whereas she is primarily spastic. A typical example is a patient referred with a footdrop who, on examination, has normal ankle dorsiflexion strength but in whom the gate cycle initiates plantarflexion spasticity. In such a case, the “weakness” often can be treated by managing the spasticity. Spasticity is a velocity-dependent increase in muscle tone (24). It is believed to be an interruption in the neural circuitry that regulates muscles, and it is a common complication of MS. Spasticity affects up to 50% of patients and can impede range of motion (ROM), hygiene, positioning, and functional use of limbs (25,26).

A word of caution: A patient may occasionally use spasticity as an aid in transfers or ambulation. Before spasticity is reduced, it is important to determine whether it harms or helps patient function. A functional assessment by a physical or occupational therapist is very helpful in making this decision.
Spasticity can be clinically assessed by the Ashworth, Modified Ashworth, Oswerty, or Tardieu scales. These scales take into account the degree of increased tone and change in ROM. More sophisticated evaluation methods include neurophysiologic and mechanical methods using multichannel EMG equipment, which can differentiate contracture from agonist–antagonist muscle activity (27). It is important to reiterate that some degree of hypertonus can occasionally improve a patient’s ability to ambulate, transfer, or stand.

**Spasticity Management**

There is a sequential approach to manage spasticity in MS patients. If one step does not resolve spasticity, subsequent steps can be added. It is useful to approach the problem in the following order.

**Nociception**

The first step in reducing spasticity involves removing the nociceptive input (28). This includes checking for urinary tract, pulmonary, or sinus infections; pressure sores; bowel obstructions; ingrown toenails; fractures; an acute abdomen; or any other noxious stimulus. All of these can contribute to increased spasticity.

**Stretching**

The second step in reducing spasticity is patient education on proper stretching and exercise routines. Because spasticity involves an interfering stretch reflex, the goal in management is to reduce the sector of the movement arc, which stimulates the stretch reflex. By stretching the tendon/muscle complex, a less-marked stretch reflex occurs. Therefore, an effective stretching program—especially of the ankle plantarflexors and knee flexors—should be the platform upon which all physical rehabilitative management rests.

Steady stretching should be done for sustained periods (e.g., minutes) at a force that is sufficient to cause 30 minutes or so of post-stretch discomfort; the stretch should take place several times a day (29). Deep or superficial heat facilitates stretch and reduces discomfort (30). Daily ROM and stretching can help prevent contractures and capsule tightness (27). EMG bio-feedback, botulinum toxin, chemical blocks, and transcutaneous electrical neural stimulation (TENS) can also be used to reduce spasticity and facilitate stretch.

**Spasmolytic Agents**

The third step in managing spasticity is the use of oral spasmolytic agents. The mechanisms of action and anatomic sites of antispastic medications are not completely understood. Some alter the function of transmitters or neuromodulators in the CNS, while others work peripherally. CNS actions include suppression of excitation (glutamate), enhancing inhibition (GABA or glycine), or both. The four most commonly used medications for spasticity and hypertonus are Baclofen, Tizanadine, Dantrolene, and Diazepam (Table 2).

Baclofen acts on the inhibitory GABA-B neurotransmitter receptors. It is more effective in reducing flexor spasms. This is the platform drug used for MS spasticity. Tizanadine another alpha-2 agonist. Its efficacy is similar to baclofen, but it produces less peripheral weakness. Side effects include extreme drowsiness (so it must be titrated very slowly), dry mouth, and hypotension. Dantrolene is a medication which
<table>
<thead>
<tr>
<th>Medication</th>
<th>Daily dosage</th>
<th>Half-life</th>
<th>Metabolism</th>
<th>Site of action</th>
<th>Patient population best suited</th>
<th>Side effects</th>
<th>Lab monitoring/notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baclofen</td>
<td>10–80 mg (in four divided doses)</td>
<td>2–6 hr</td>
<td>Kidney, liver</td>
<td>CNS, GABA-B inhibition</td>
<td>Best in spinal forms of spasticity and flexor spasms (MS, SCI), CP, stroke</td>
<td>Generally mild: somnolence, fatigue, constipation, nausea, vomiting</td>
<td>If discontinued, use slow taper, LFT monitoring suggested</td>
</tr>
<tr>
<td>Tizanadine</td>
<td>4–36 mg (in 1–3 divided doses) slow titration recommended</td>
<td>4 hr</td>
<td>Liver</td>
<td>Central alpha–2 agonist, glycine facilitator</td>
<td>MS, SCI, CP, stroke, TBI</td>
<td>Orthostatic hypotension, drowsiness, dry mouth, dizziness, MS patient prone to muscle weakness</td>
<td>LFT monitoring suggested</td>
</tr>
<tr>
<td>Dantrolene</td>
<td>25–100 mg (in four divided doses) 4–15 hr (after oral dose)</td>
<td>Liver</td>
<td>PNS inhibits Ca release at sarcoplasmic reticulum</td>
<td>TBI, stroke, MS, SCI</td>
<td>Most hepatotoxic, postural instability, slurred speech, diarrhea</td>
<td>Preferred in TBI patients since no BBB passage</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td>2–40 mg (in divided doses) 20–80 hr</td>
<td>Liver</td>
<td>CNS, facilitates GABA inhibition</td>
<td>MS, SCI, CP, stroke</td>
<td>Sedating, memory impairment; not usually recommended in TBI patients</td>
<td>If discontinued, use slow taper, patient should not consume alcohol when using diazepam</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; CP, cerebral palsy; LFT, liver function tests; MS, multiple sclerosis; PNS, peripheral nervous system; SCI, spinal cord injury; TBI, total body irradiation.*
works peripherally at the excitation–contraction coupling of muscle fibers and causes the inhibition of calcium ion release from the sarcoplasmic reticulum. Dantrolene is preferred in brain injury and cerebral palsy patients due to its peripheral site of action and decreased amount of cerebral absorption but is not the drug of choice for MS, as it increases weakness. Liver function tests should be monitored due to a small risk of hepatotoxicity. Diazepam acts centrally on the GABA-A receptors and facilitates GABA-mediated inhibition in the brain and spinal cord. Baclofen and diazepam are centrally acting medications with similar side effects, however, they are more pronounced with diazepam. These include sedation and memory impairment. Because these symptoms are also produced by MS, it is rarely used in this disease.

The art of determining the dose of spasmolytic agent should be based upon a functional assessment rather than the elimination of spasticity on physical examination in the clinic. Experience has indicated that the reduction of hyperreflexia is not the goal; the goal should be to reduce ankle clonus (but not eliminate it) to 3 to 5 beats (31). The use of too much spasmolytic agent will result in the “spaghetti-legs” syndrome, where patients’ function is decreased (32).

**Nerve Blocks**

The fourth step in managing spasticity is the judicious utilization of selected local nerve blocks.

Botulinum toxin (Botox®) and dilute phenol injections are used for medication-resistant spasticity. Blocks can also be used in conjunction with spasmolytic medications. The effects can last from months to years depending on the agent used. The purpose of the block should be considered prior to deciding which agent to use. Blocks can be done on a mixed nerve, motor nerve branch, or motor points. Phenol is more commonly used to target large nerves and muscle groups while botulinum toxin is typically used in smaller muscles.

**Baclofen Pump and Surgery**

The final step in managing spasticity involves surgical procedures. The most commonly employed surgery is the implantation of the baclofen intrathecal pump (33). This excellent procedure allows for variable amounts of baclofen to be infused intrathecally at various times throughout a 24-hour period. Because it is a targeted delivery, a much smaller amount of baclofen can be used than is required systemically (micrograms rather than milligrams), resulting in reduced systemic effects. This procedure, however, is of use only for lower limb spasticity; there is little effect on upper limb spasticity.

Other surgery is reserved for use after all of the above methods fail or if a joint exhibits an intra-articular contraction. Surgery can be used to correct deformities, increase comfort and bed positioning, and improve function and cosmesis. Some procedures include:

1. Achilles tendon lengthening (TAL). This procedure is typically used to correct severe plantar flexion deformity. It is indicated if stretching has not achieved this and correction of the deformity is deemed important to improve the patient’s function.
2. Split anterior tibial tendon transfer (SPLATT) (often combined with Achilles tendon lengthening). This procedure is used to correct an equinovarus deformity when a TAL is not sufficient.
3. Subtalar fusion or triple arthrodesis. In rare cases this procedure can be used to improve cosmesis and decrease clonus.
4. Adductor tendon section. In bed-bound patients this procedure relieves hip adductor spasms to allow better perineal hygiene.
6. Posterior rhizotomy. This procedure is used for spasticity management in rare cases of intractable spasticity.

Exercise

A frequent clinical observation in MS patients is that they complain of tripping and falling by catching a toe, but on examination have excellent ankle dorsiflexion, although the toe of their shoe may show evidence of scuffing. Such patients should be examined in a dynamic setting and observed ambulating over a sufficiently long distance to bring on symptoms. This phenomenon may be analogous to Uhthoff’s syndrome in the visual pathway, and might be termed a “motor-Uhthoff’s phenomenon.” It may be that activity results in a sufficient increase in core body temperature or chemical change to produce conduction block in neural pathways supplying ankle dorsiflexors. Treatment might include a trial of 4-aminopyridine or pyridostigmine (Mestinon\textsuperscript{1}) supplemented by an ankle foot orthosis (AFO) and/or a cane for long distance ambulation. In other patients, a simple solution such as coating the toe of a rubber-soled shoe with liquid plastic will reduce the chance that the toe will “catch” on the floor and cause the patient to fall.

Another circumstance in which an MS patient may fall in the absence of any objective ankle weakness is during multitasking. This is usually a greater problem for patients with significant brain atrophy. As such patients worsen, there occurs a spread of the cortical-neural territory activated in performing a motor act, an observation that has been confirmed by fMRI studies (17). When patients are at this stage, they need to consciously focus on their gate. When distracted, as may occur when talking with a companion, the complex motor activities required for a stable gate may not be adequately sequenced; they are less “automatic” and require more voluntary activity. Even though it may appear that these patients are tripping because of ankle weakness, an AFO may not be the best solution and may interfere with functions throughout much of the day. They may be benefited more by broadening the base of support with a cane.

Recognizing this difficulty in multitasking may lead to a paradigm shift in the understanding and management of MS; Deficits occurring in real-world experiences may not appear when each affected system is examined in isolation (34,35). An MS patient with “weakness” must therefore first be assessed for spasticity and clonus, a motor-Uhthoff’s phenomenon, and multitasking interference. When all of these have been treated and true weakness is still present, a substitutive treatment needs to be employed.

We have shown that progressive resistive exercises (PREs) can improve strength in patients with MS (36). Increasing muscle strength with resistive exercise requires the development of intramuscular tension. Although MS patients are often unable to generate levels of tension typically used for optimal strength training, both mildly and severely affected patients are able to generate sufficient levels to increase strength. As would be expected, mildly affected patients can generate a greater increase in strength, although both can achieve an increase in function (37). Consequently, we recommend PREs in managing MS weakness.
Bracing

If the above are not sufficient to improve function, some type of bracing may be needed. Patients with significant weakness need a stabilizing standard AFO. If there is significant spasticity and clonus, the classic fixed-ankle plastic AFO (PAFO) is the desirable orthosis. When some degree of ankle movement is desired, an articulated AFO may be a better choice. Our research has indicated that in mild paresis (or even in motor-Uhthoff’s phenomenon) an electrical stimulation AFO is preferred by patients (38).

One of the other characteristics of management of weakness in patients with MS is that one solution may not be ideal for all situations. For example, a patient might do very well using a cane and/or AFO for short distances, but require a wheelchair for long-distance ambulation. A patient’s function needs to be improved by using the optimal ambulation aid for any given occasion. Furthermore, because MS typically is a progressive disease, a successful rehabilitation strategy needs to be revisited over time to determine whether additional measures need to be employed.

BODY COOLING

Heat intolerance is a common problem faced by up to 80% of MS patients. Cooling can provide symptomatic relief to these patients. This has led to the manufacturing of cooling vest and head-vest garments. There are three types of “passive” cooling vests commercially available: gel pack, phase change, and evaporative (39). A brief description of the three is given below:

1. Gel-ice (example: Steele vests, Steele Inc., Kingston, WA, U.S.A.): A mixture of starch and water that has a similar cooling property as ice when frozen
   A. Advantages: no leakage; several test reports have proven core body temperature reduction; maximum cooling power (40).
   B. Disadvantage: does require freezing.

2. Phase change material: Paraffin material that freezes between approximately 55°F to 65°F; can be cooled in ice water by conduction or in a freezer via convection.
   A. Advantages: moderate cooling power (40); cools at a comfortable temperature and therefore decreases the risk of reflexively increasing the core body temperature secondary to peripheral vasoconstriction. The phase change materials cool to a temperature above dew point and therefore will not condense (41).
   B. Disadvantages: flammable if fluid leaks; heavy (4–7 lbs.).

3. Evaporative material: Three layer composite that evaporates water that is stored in the center layer through wicking.
   A. Advantages: light weight, low profile, low cost, disposable.
   B. Disadvantages: light cooling power (40); it is disposable and must be purchased on a monthly basis.

Another type of cooling uses an “active” process. A mixture of distilled water and propylene glycol is circulated through a refrigerating unit (either electrical refrigeration or ice-filled container) and tubes embedded in a vest. The cool fluid
extracts heat from the patient and dissipates it in the unit. Examples of this active system are those made by Life Enhancement Technologies (LET, Mountain View, California, U.S.A.).

A study has been conducted in MS patients using several types of cooling vests: the LET active cooling garment, the MCS system, which is a vest that is activated by refrigeration, and the Steele vest, which needs to be activated by freezing (42). It was found that the LET active and Steele passive vests produced similar, significant cooling effects of oral and ear canal temperatures. Skin temperature decrease occurred in all three groups during cooling period. However, the Steele vest group skin temperature continued to decline during the recovery period. The study concluded that the LET active vest produced the most patient improvement measured by energy level, muscle strength, and cognitive ability.

The authors concluded that picking the best cooling vest often depends on patient profile. An LET active vest is more expensive and requires more effort to use, but allows more control over the amount of cooling done. The MCS and Steele vests are passive and therefore cost less, require no power source or heat sink, and are easily portable. The passive vests provide no control over temperature settings and, as seen in the study, the initial cooling period causes peripheral vasoconstriction and an increase in core body temperature.

Another study (43) compared the effect of active versus sham cooling in ten MS patients with EDSS scores between 3.5 and 6.5. Active cooling resulted in significant improvement in fatigue (Short Fatigue Questionnaire), postural stability with eyes closed, and lower limb muscle strength.

We have studied heat extraction in MS subjects using an active cooling system with a one-group, two-treatment, repeated-measures, within-subjects design (44). The treatment (temperature) condition was randomly ordered and had two levels: sham body cooling (SC; 26.5°C) and active body cooling (AC; 7°C). Seventeen HSMS subjects completed the experiment. Subjects were fitted with a Mark I Medical active Cooling Garment, Mountain View, CA, U.S.A. The HSMS wore the garment for 60 minutes while resting comfortably in a chair. Body core temperature, heart rate, and brachial blood pressure were monitored every five minutes.

There was a significant improvement after the treatment condition (AC) in several measurement domains: strength (quadriceps), endurance task (leg cycling), dynamic balance task (tandem gait), single leg standing balance, and ambulation velocity. We concluded that heat extraction enhances the ability to do repetitive activities in HSMS patients.

But what is the mechanism by which cooling improves the function of MS patients? Leukocyte nitrite concentration, which is reflective of nitrous oxide (NO) production, has been measured before and after active cooling. NO is a diffusible gas that can enter the CNS and block conduction in demyelinated axons. MS patients had significantly higher leukocyte nitrite concentrations at baseline when compared to 12 healthy volunteers. Active cooling resulted in significantly lower concentrations of NO compared to sham cooling. This suggests that the symptomatic relief of cooling garments may be related to a decrease in leukocyte nitrite production as opposed to the commonly held belief that the neural safety factor of partially demyelinated nerves is improved by CNS cooling. Other chemical and hormonal changes may be present. Bowen et al. noted a significant rise in norepinephrine (probably related to vasoconstriction) as well as a modest decrease in thyroid stimulating hormone (TSH) (45).
In summary, heat extraction or cooling does have a beneficial effect for many MS patients. The mechanism is still unknown, but it clearly involves more than just the reduction of cord and brain temperature.

**ATAXIA AND TREMOR**

Tremors can be quite debilitating for patients because they interfere with the ability to perform basic ADLs such as feeding and grooming. Alusi et al. (46) studied the type and severity of tremor in MS patients. They evaluated 100 patients and measured the severity of tremor using finger tapping and nine-hole peg tests, and the subject’s ability to draw an Archimedes spiral. Fifty-eight patients had a tremor with 20 subjects reporting asymptomatic tremors. Affected regions were arms (47), legs (10), head (9), and trunk (7). Each case of tremor reported was of an action type (postural, kinetic, or both). The authors did not observe any true rest tremors. Exacerbating factors included anxiety, hot baths, and excessive physical exertion. While various combinations of body parts were involved, the most common included bilateral arm involvement. The most common type of tremor was a coarse distal tremor of the arms.

When comparing the tremulous and nontremulous patient groups, some significant differences between the groups were EDSS score and wheelchair reliance; the tremulous group had higher EDSS scores (6.0 in the tremulous group versus 5.5 in the nontremulous group) and were more likely to be wheelchair dependent. Twenty-seven percent of the subjects had a tremor-induced disability and 10% had an incapacitating tremor. The authors found no correlation between MS disease duration and tremor severity. They commented on an interesting correlation between MS patients with or without tremor and the presence of a family history of tremor. Seven percent of the MS patients reported a positive family history of tremor. This raises questions as to whether some tremor seen in an MS population may be a result of the disease, a genetic predisposition to tremor, or both.

Treatment of tremor can involve pharmacologic, rehabilitative, or surgical intervention. A key step is to correctly diagnose the type of tremor present as treatments vary according to type. Resting tremors are not voluntarily activated and occur in body parts with complete support. Action tremors occur with voluntary movements and include postural, kinetic, isometric, and intention tremors. Agents that have been used with mixed success include carbamazepine, propranolol, tetrahydrocannabinol, clonazepam, and isoniazid (46, 48).

In severe cases, upper-limb intention tremors can be an enormous problem, often resulting in an almost insurmountable disabling condition. In spite of the encouraging reports of medical and surgical interventions, perhaps the most effective rehabilitation strategy is the employment of a heavy resistive weight on the distal limb, which may help reduce the excursion of the extremity (49).

For severe cases of tremor, stereotactic thalamotomy and deep brain thalamic stimulation (DBS) have been performed. Accurate diagnosis and patient selection greatly influence outcome. DBS is indicated for patients with relatively stable disease and disabling upper limb tremor (50). With careful patient selection, unilateral thalamotomy has been used with success rates between 69% and 96% (51). With both of these procedures, there is a 20% risk of tremor recurrence within a year. The target area for neurosurgical treatment of tremor is the nucleus ventralis posterior, which is
the cerebellar input nucleus of the thalamus. More recently, the area of interest is the nucleus ventralis oralis posterior, which is the basal ganglia output center. This suggests that MS tremors may be generated from the basal ganglia despite the cerebellar appearance of the tremor (52). Complications from thalamotomy include worsening of gait, hemiparesis, confusion, and lethargy (47).

An exciting new development to treat tremors, dysmetria, and weakness are virtual reality systems that work based on the adaptive ability of neuroplasticity in the brain (53). Haptic systems are currently being developed that are cued by the patient’s environment. The system then provides patients with cues and provides “force corridors” to help guide the patient’s wrist and hand movements. There is ongoing research on developing sensory augmentation for visual and proprioceptive loss (53,54).

At the present time, the most practical management of ataxia involves either broadening the base of support with a cane or crutch or, more optimally, a walker for greater stability. When this is not sufficient, the avoidance of bipedal ambulation is the goal and patients need to be taught to function in a scooter or wheelchair. MS patients with ataxia like three-wheeled electric scooters as they are typically not weak but have problems with motor control. These scooters are relatively easy to disassemble for the trunk of a car and do not require the purchase of a special van.

SENSORY LOSS AND PAIN

We have surveyed the prevalence, intensity, interference, and biopsychosocial correlates of pain in a large community-based sample of 442 persons with MS. Forty-four percent of respondents reported persistent bothersome pain in the three months prior to completing the survey. About 25% of participants with pain reported severe pain (score of 7–10 out of 10), while 51% of those with pain rated the interference of their pain with daily activities as none to minimal. Twenty percent reported severe interference in activities as a result of pain. MS illness severity, marital status, and self-ratings of overall health were significantly associated with pain-related interference with activities. Approximately a fourth of this sample described having a chronic pain problem that was characterized by severe pain intensity and significant pain-related interference with activities (55).

Pain and sensory loss are thought to represent disease of the dorsal cord and are best managed by anticonvulsant medications rather than analgesics. It is important to identify the cause of the pain. If the pain is neuropathic—classically a sharp, lancinating type of pain—anticonvulsants are the treatments of choice. Because MS patients have spasticity and weakness, they are also at greater risk for musculoskeletal pain, which should be treated as one would manage any painful musculoskeletal condition: nonsteroidal anti-inflammatory medications and other standard rehabilitation techniques, bearing in mind the caveat that patients with MS are often too weak to benefit from exercises traditionally prescribed for management of this type of discomfort. Consequently, creative bracing can often be substituted for weak muscles and provide a satisfactory treatment solution. Pain will be discussed in greater detail in Dr. Shapiro’s chapter (chap. 10) in this book.

Sensory impairment, although a common MS symptom, is not directly treatable. It is often just a fact of MS life: annoying but not problematic.
DEPRESSION

A survey of 739 patients from Western Washington was conducted by our MS center. Almost 42% of these patients suffered from clinically significant depression (as defined by the Center for Epidemiologic Studies Depression Scale) (56). The study reported two interesting findings: (i) Depressive symptoms are more likely to occur as the disease worsens, and (ii) There is an additional period of depression shortly after diagnosis. Other factors consistent with more severe depressive symptoms include younger age, less education, and lack of social support.

Antidepressant medication use in MS patients is harmful. These medications often have untoward side effects, including fatigue. Our center is conducting two separate studies: one study to evaluate the effects of exercise in clinically depressed MS patients; and the other to determine the efficacy of an [selective serotonin reuptake inhibitor (SSRI)] antidepressant. Because of the prevalence of depression in persons with MS, finding effective treatments will substantially contribute to patient QOL.

COGNITIVE IMPAIRMENT

For a number of reasons, cognitive impairment may be considered the most severe effect of MS. It is cognition that makes us human and allows for maximal human function (57). Ongoing disease progression leads to cerebral atrophy and impaired cognitive function. Unlike physical function, cognitive impairment does not remit (58). The most common deficits in MS patients are in memory, learning, attention, and information processing (58). Severe disability and cognitive impairment are predictors of loss of employment, decline in standard of living and withdrawal from social and leisure activities, and are strong indicators of stress among relatives (61).

Unfortunately, however, cognitive impairment may be among the least recognized of symptoms, as it is a “hidden” disability. It is often not recognized in the typical office visit because verbal function tends to be preserved. In most cases, it is the higher cortical function of integrative thinking—so-called executive function—that is most affected. When told of the severity of the deficit, a physician or health-care provider might be surprised and respond, “but she talks so well...” (34).

In populations of MS clinic patients, up to two-thirds are reported to be cognitively impaired (60). However, this may be skewed because of the more severe disease seen in clinic populations. In the general MS population the prevalence may be lower; Rao et al. (61) reported cognitive impairment among 43% of MS patients in the community.

Accurate assessment and quantification requires neuropsychological testing. Complete testing is lengthy and expensive, but may be required for a full assessment of the patient’s cognitive status so that targeted treatments (e.g., memory book) can be applied. An excellent review can be found in an article entitled “Neuropsychological Evaluation & Treatment of Multiple Sclerosis: The Importance of a Neuro-Rehabilitation Focus” by Pepping and Ehde (62).

One of the most effective ways to delay or prevent cognitive decline is to treat the disease as soon as possible. There is controversy as to whether medications such as Aricept® can be useful. An early study of 5 mg a day failed to demonstrate
statistical significance, although a more recent and limited study using twice that
dose suggested it may be helpful (60,63).

**GENERAL FITNESS**

**Aerobic Exercise**

MS patients often avoid exercise due to the increased body temperature generated by
physical activity or in order to conserve their energy for other tasks. Limiting exercise
activity can occasionally lead to greater weakness, fatigue, and health risks (64). Petajan et al. (65) studied the effect of exercise in MS patients with EDSS scores \( \leq 6 \), measuring several exercise and psychological variables. Subjects in the exercise group participated in 40 minutes of supervised training programs three times a week. The authors concluded that aerobic activity (training intensity of 73% maximum heart rate) resulted in significantly improved cardiovascular fitness for the exercise group compared to the nonexercise group. Skinfold thickness and triglyceride levels were significantly decreased. Exercise did not increase the incidence of exacerbation. Long-term carry over effect of the treatment and change in disease course were not studied.

MS patients with specific disorders such as contractures or motor deficits may
require assistance from a physiatrist in planning a treatment program. The program
should involve active and passive ROM exercises, specific muscle strengthening,
ADL training, and active recreation in a structured program (65).

It is important to know what types of exercise may be detrimental to patients
with MS. A study of 18 MS patients with EDSS scores \( \leq 4 \) was conducted to evaluate
any gait and ROM changes that might occur after six months of standard aerobic
exercise (66). All participants engaged in 30 minutes of arm/leg cycle ergometry three
times a week at 65% to 70% age predicted maximum heart rate. The study found that
gait did not improve with cycle ergometry. More specifically, ankle angle became more
plantarflexed, knee ROM decreased, and hip flexor tightness increased. Hip abduc-
tion, adduction, and external rotation with the knee extended increased, however, this
was offset by the increased Thomas angle (measurement of hip flexion ROM).

Exercises should be written with the patient’s current functional status and
goals in mind to ensure the best chance at functional improvement. In all exercises,
patients should be cautioned to avoid overheating.

**Yoga**

Yoga has become more popular in western civilization over the past decade. It is a
low impact, aerobic exercise that aims to improve mental and physical health. There
are several forms of yoga, one of them being Iyengar. In Iyengar yoga, a person goes
through a series of stationary positions that utilize isometric contraction and relaxa-
tion of different muscle groups to form specific alignments.

The effect of Iyengar yoga practice in MS patients was studied in a six-month,
parallel group, randomized, single-blinded, controlled clinical trial comparing patients
in a yoga, aerobic exercise, and control group. The study enrolled a total of 69 patients
with MS with EDSS scores ranging from 1 to 4. Fifty-seven patients completed the
study. Outcome measures included cognition, alertness, mood, fatigue, and QOL.
Two of the study scales used included the SF-36 and Multidimensional Fatigue Inven-
tory (MFI).
Both the yoga and exercise programs improved fatigue as assessed by the MFI (general fatigue) and the SF-36 (energy and vitality) scales. Neither the yoga nor aerobic exercise group demonstrated significant improvement over placebo in alertness, attention, or cognition (67).

Aquatic Exercise

Aquatic exercise is encouraged by the National Multiple Sclerosis Society (NMSS) due to the low impact, cold temperature, and gravity-effect reducing properties of exercising in water. Patients who cannot stand or ambulate on ground are able to do so in water and thereby increase their flexibility. Additionally, there is the mental satisfaction and improved QOL that should not be discounted. There are several “testimonials” online and in pamphlets of patients with MS who report the mental and physical gains they made through aquatic programs. The NMSS runs aquatic exercise programs across the country. Those patients interested are advised to contact their local chapter for further information.

There are few published papers on the benefits of aquatic exercise in MS patients. One study included 10 MS patients (68). Length of disease, EDSS values, and treatments that the subjects were or were not stated. The study evaluated the effect of isokinetic exercises on upper and lower limb torque and force after a 10-week period of exercise. No clear benefit was shown. However, there was also neither an adverse effect nor a decline in function or strength.

ASSISTIVE TECHNOLOGY

Assistive technologies (ATs) include any item, piece of equipment, or product system, whether acquired commercially off-the-shelf, modified, or customized, that is used to increase, maintain or improve functional capabilities of individuals with disabilities (70). Commonly used ATs are canes, walkers, grab bars, tub benches, and wheelchairs. Less common but equally useful devices include modified utensils, weighted objects, jar openers, computer screen readers, and augmentative communication devices.

AT devices specific to MS patients include:

1. Visual aids, which include an eye patch for diplopia, large print texts, or magnifier glasses to enlarge texts. For those individuals who cannot read at all, audiotaped books are an option. To increase the ease of reading, nonglare paper is a simple solution. Verdana font in 10 to 12 point is the preferred font to decrease visual fatigue.

2. Programs to aid patients with cognitive impairments in completing tasks. Programs, such as the PocketCoach®, AbleLink Technologies, Inc., Colorado Springs, CO, U.S.A. and personal digital assistants (PDAs), like the Palm Pilot®, PalmSource, Inc., Sunnydale, California, U.S.A., provide auditory cueing about sequential steps. The PocketCoach is compatible with Palm and Windows platform software and PDAs are available at many office supply stores.

3. Speech augmentation aids, which are useful for patients with severe hypophonia. Such patients are cognitively capable of discourse, but have such severe vocal motor impairment that they may only be able to do little more
than whisper. These aids are speech amplifiers and are especially useful in a loud environment. For such patients, a specialized telephone can amplify their voice to enable a telephone conversation.

For clinicians interested in providing AT devices to their patients, a referral to a vocational counselor or AT specialist is recommended. If this is not available, a physical, occupational, or speech-language therapist, or a therapeutic recreation specialist can provide options as well.

Funding for AT can present a challenge. Patients with private insurance need to contact their respective companies for information on coverage. Although Medicare and Medicaid Part B cover durable medical equipment, state-to-state laws on coverage of specific items may vary. Veterans can obtain assistance through their local Veteran’s Affairs AT specialist or social worker. Patients are encouraged to look into their local and state AT funding agencies.

Additionally, local chapters of the NMSS and other groups may be able to provide assistance for those in need.

VOCATIONAL ISSUES

Often, the summation of an MS patient’s somatic, cognitive, and affective impairments results in difficulty in sustaining employment. Persons with MS are employed at a much lower rate than would be expected from a cursory examination in the physician’s office (32). Employment statistics indicate that while one might expect the relatively high levels of education among individuals with MS to correspond to a high employment level, in fact only 20% to 30% of individuals with MS are employed within five years of diagnosis (70), although 40% of those unemployed say they would like to return to work (71).

Although there are a variety of reasons for this, the treatment is clear: optimally manage the patient’s disease and symptoms, recognize the contribution of cognitive and physical difficulties, and refer the patient to a vocational counselor or the state Vocational Rehabilitation program. Such referrals should not wait until after the patient has lost her job. They should be initiated earlier in the disease, when the first indication of impaired job performance is noted.

CONCLUSION

MS care may be looked at as having three stages. Several decades ago MS was considered to be a disease of ambulation. Indeed, the widely used EDSS, developed by Dr. Kurtzke, is mainly an ambulation measure at higher disability levels (72). This might be considered phase I: MS was a disease of ambulation.

The next phase, phase II, identified many abnormalities other than the obvious ambulation impairment in patients with MS, including cognition, memory, and depression. The field of MS management was advanced as studies were carried out of these and other symptomatic systems.

More recently, we may be entering phase III, where total disability is seen as more than the sum of each impairment. Evaluating the impairment in each system, and adding them together, often does not begin to describe the severity of the disability seen in the person with MS. Patients tend to do better on evaluation of individual
systems than they do when all systems are forced to perform simultaneously—as required for the multitasking of life. The gestalt is what is important (34).

In summary, multiple sclerosis is a complex disease affecting the very essence of what makes us human. Its progression results in a constantly moving target for interventions. Because it affects multiple portions of the brain, cerebellum, brainstem, and spinal cord, its protean symptoms present a challenge for management. Rehabilitative services are what the patient requests and must be an important component of any satisfactory management strategy (12).

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REFERENCES


INTRODUCTION

Most patients with multiple sclerosis (MS) experience relapses characterized by acute or subacute neurological dysfunction lasting days to several weeks followed by a remission with partial or complete resolution of neurological dysfunction. Attacks may occur as part of diverse demyelinating syndromes: clinically isolated syndromes [e.g., isolated optic neuritis (ON), myelitis, or brainstem syndromes], relapsing–remitting MS (RRMS) or secondary progressive MS, or one of the atypical demyelinating disease variants (Marburg variant, tumefactive MS, severe monophasic disorders such as complete transverse myelitis and neuromyelitis optica). The goals of acute treatment are reversal of neurological disability sustained from an attack, arrest of rapidly deteriorating neurological dysfunction, and restoration of function. This may be distinguished from the goals of long-term therapies, such as interferon beta and glatiramer acetate, which are not known to alter the course of an individual attack and its sequelae, but rather to reduce the probability of subsequent clinical and subclinical attacks.

The study of acute treatments for MS presents many challenges. First, most attacks are self-limiting and improve either spontaneously or after a course of corticosteroids. Documenting restoration of function attributable to the effects of acute treatments alone is therefore difficult. Second, the wide spectrum of neurological deficits observed in patients with prototypic MS precludes the use of widely accepted clinical composite measures such as the expanded disability status scale (EDSS) for clinical trials targeting the diverse spectrum of attacks. Third, the broad clinical heterogeneity of recurrent demyelinating disease may be indicative of different underlying pathophysiologic mechanisms. An example is neuromyelitis optica which may have a different pathophysiologic mechanism than prototypic RRMS; current evidence suggests that neuromyelitis optica may be a predominantly humorally-mediated disease. Finally, the coexistence of ongoing progressive demyelination can undermine the benefit of acute treatment used in patients experiencing relapses.

Short courses of high-dose corticosteroids are generally regarded as first-line treatment for disabling attacks. Corticosteroids are believed to shorten the course...
of an attack but not alter the final outcome. However, there is some evidence for a beneficial effect of long-term use of steroids in RRMS and secondary progressive MS. Despite the universally accepted role of pulsed high-dose methylprednisolone (HDMP) for acute attacks, the optimal dose and efficacy of this approach has not been established, and the available data require further scrutiny.

Intravenous immunoglobulin (IVIg) may be valuable as a long term “disease-modifying” treatment in RRMS. Other than uncontrolled observational data, the evidence for its use in treatment of acute relapses is lacking. In addition, it has not been shown to benefit patients with secondary progressive MS.

Treatment of catastrophic attacks marked by rapid development of severe disability and poor response to corticosteroid treatment requires a different approach. This group of patients may be referred to as “catastrophic MS” and represent a small fraction of patients experiencing an acute attack, particularly those with severe deficits occurring in the context of neuromyelitis optica or Marburg’s fulminant MS, who have partial or no response to corticosteroids. The acute clinical presentation of patients with catastrophic MS may be divided into two categories: (i) acute fulminant MS, characterized by severe acute attack with the development of severe disability over less than one month and (ii) rapidly worsening MS, characterized by rapid accumulation of major disability in a step-wise or continuous fashion over less than six months, caused by frequent relapses or continuous progression of repetitive and multifocal inflammatory demyelination refractory to conventional treatments (Fig. 1).

Patients presenting with catastrophic attack of idiopathic inflammatory demyelinating disease refractory to steroids benefit from therapeutic plasma exchange (TPE) after exclusion of other mimicking disorders. In a recent double-masked, randomized, sham-controlled, crossover study to evaluate TPE as monotherapy in this setting (1), patients with acute severe attacks experienced dramatic improvement in a variety of demyelinating syndromes within days. On the basis of these results, TPE is now regarded as a category II indication for treatment of acute severe demyelinating attacks by the American Society for Apheresis (2).

Some patients follow a pattern of rapidly worsening inflammatory demyelination with clinical or radiographic evidence of ongoing inflammation either in a

![Figure 1](image) Catastrophic demyelinating disease: (A) Acute fulminant presentation, characterized by severe acute attack and development of severe disability over less than one month that is refractory to corticosteroid treatment. (B) Rapidly worsening presentation, characterized by rapid, typically step-wise accumulation of major disability over less than six months.
relapsing or in a secondary progressive phase of their disease. These patients are appropriately treated with immunosuppressive agents such as cyclophosphamide (CTX) or mitoxantrone (MTX) (Fig. 2). Given the lack of absolute criteria for rapidly worsening MS, recognition of this subgroup of patients remains a challenge. Moreover, rapid recovery of neurological deficits may not occur following immunosuppressant therapy. Each of these therapeutic options will be discussed separately in the following sections.
TREATMENT WITH CORTICOSTEROIDS

It is common practice to prescribe HDMP for treatment of acute relapses. MP binds to albumin and glucocorticosteroid-binding globulin at low-doses. The blood–brain barrier has limited permeability to these protein complexes (3). HDMP, however, saturates the binding proteins and has higher free levels in serum, thereby facilitating its crossing the blood–brain barrier.

MP reduces the inflammatory response through different mechanisms. It acts to inhibit synthesis of proinflammatory molecules such as immunoglobulins, cytokines, and growth factors; it stabilizes the blood–brain barrier, and thereby decreases infiltration of inflammatory cells. Cell membranes are highly permeable to MP. It exerts its effects on the membrane-bound and intracellular receptors to downregulate growth factor and cytokine gene expression by inhibiting transcription through the DNA-binding domains of the glucocorticoid receptors (4,5). MP also reduces inflammatory cellular infiltration through the blood–brain barrier. It limits mononuclear cell transmigration through endothelial cell monolayers by reducing adhesion molecule expression; it also has direct effects on the endothelial cells and the peripheral blood mononuclear cells (6). MP may also promote apoptosis in peripheral blood leukocytes, thereby limiting the autoimmune process (7).

Corticosteroids and adrenocorticotrophic hormone (ACTH) have been used to treat MS exacerbation since the 1950s. ACTH was regarded as treatment of choice for the acute relapse after a clinical trial, in 1970, showed better recovery following ACTH versus placebo for relapses of MS (8). Subsequently, three randomized clinical trials compared ACTH with intravenous (IV) HDMP (9–11). A summary of the clinical trials, on the use of corticosteroids in acute demyelinating exacerbations, is provided in Table 1. They were small trials of relatively short duration and lacked statistical power. None showed significant difference in efficacy between the two treatments nor suggested that MP may be better tolerated than ACTH. Recent direct comparative trials have not shown significant difference between ACTH and MP in the degree and rate of recovery after an exacerbation (11,20).

In the ensuing years, three randomized, double-blind, placebo-controlled trials were completed to assess the benefit of IV or oral HDMP for relapses in MS (12–14). All three showed a statistically significant benefit of HDMP compared to placebo in the degree of recovery from relapses in MS, despite the small number of patients and short duration of the trials; there was a greater short-term reduction in the EDSS with the use of HDMP when compared with placebo. A meta-analysis showed a clinically meaningful pooled treatment difference (improvement in EDSS of 0.76) (21) and provided strong support for the use of HDMP for acute relapses.

Subsequent clinical trials were carried out to determine the optimal formulation, dose, and route of administration of corticosteroids. Oliveri et al. (15) directly compared IVHD and low-dose (LD) MP in a double-blind randomized trial and found no statistically significant difference in mean EDSS in short-term follow up, despite lower magnetic resonance imaging (MRI) activity in the group receiving HDMP. La Mantia’s double-blind randomized study of 31 patients compared HD and LDMP and dexamethasone (DX). DX and HDMP had similar efficacy, although there was a trend toward lower relapse rate in the group receiving HDMP; the group receiving LDMP had a worse short-term outcome, earlier clinical reactivation, and a higher relapse rate (16).

The conclusion from the study of La Mantia et al. (21), that an increase in disease activity may occur after LD therapy, was also suggested by the Optic Neuritis
Treatment Trial (ONTT), a large 15-center clinical trial. In this study, 457 patients with ON, either associated or not associated with established MS, were randomized into three treatment groups within eight days of symptoms onset: (i) oral prednisone alone (1 mg/kg every day) for 14 days (oral treatment group); (ii) IV MP sodium succinate 250 mg four times daily (1000 mg/day) for three days in hospital, followed by oral prednisone (1 mg/kg every day) for 11 days as outpatient (IV treatment group or IVMP); or 3) oral placebo for 14 days (placebo group) (22–24). The aim of the study was to determine the rate of recovery and complications of therapy. There was no significant difference in the long-term visual outcome in the three treatment groups at six months or beyond. However, the group receiving IVMP achieved a significantly faster visual recovery when compared with placebo in the first 30 days. The group receiving oral prednisone did not achieve a statistically significant improvement in the rate of visual recovery when compared with placebo. In addition, higher rate of recurrent ON was noted in the latter group at two and five years of follow-up.

The ONTT preceded La Mantia’s study (16) and corroborated the implications of increased risk of exacerbation after LD steroid treatment. However, Barnes et al. (17) demonstrated no significant difference in the risk of new exacerbations between IVHDMP and oral LDMP treated patients during a follow-up interval of six months. Pooled data obtained from Barnes et al. (17) and La Mantia et al. (16) was analyzed in a meta-analysis, and failed to show clinically significant difference between HD and LD treated groups (21).

The ONTT also suggested that clinically definite multiple sclerosis (CDMS) occurred less frequently in follow-up of the IVMP treatment group, members of

<table>
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<tr>
<th>Study</th>
<th>ACTH</th>
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**Table 1** Clinical Trials of Different Types of Corticosteroid Treatments for Multiple Sclerosis Relapse

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<tr>
<th>Study</th>
<th>ACTH</th>
<th>PO</th>
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<td>Oral = IVMP</td>
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*aIVMP 2000 mg/day versus IV MP 500 mg/day.

*bIVMP 1000 mg/day versus PO MP 48 mg/day with a tapering 3-week course.

**Abbreviations:** PO, oral; IV, intravenous; HD, high-dose; ACTH, adrenocorticotrophic hormone; MP, methylprednisolone; DX, dexamethasone; LD, low-dose; ONTT, optic neuritis treatment trial.
which had not been previously diagnosed with MS and were followed for two years, as compared with the two other groups. This effect was primarily observed in patients with abnormal MRI scans at study entry who have a higher event rate. The potential therapeutic effect of IVHDM was no longer evident by third year of follow-up (24). There was no significant difference among treatment groups after five years of follow-up in either the rate of development of CDMS or the degree of neurological disability among those patients in whom CDMS had developed (25,26).

The results of ONTT had a significant impact on practice parameters followed by neurologists and ophthalmologists. On the basis of this evidence, oral prednisone alone is not recommended for treatment of acute demyelinating optic neuritis. Since treatment was not shown to alter the long-term outcome, the decision of whether to treat or not with HDMP usually depends on nonevidence-based factors such as quality of life, disability, or visual function of the contralateral eye (24). It has become a common practice to treat acute ON with IVMP (500 to 1000 mg) for three to five days, when the visual acuity is worse than 20/50 in the affected eye, when the recovery from previous attacks has been poor, or when there is significant pain with eye movement. Many clinicians administer a tapering course of prednisone following IVMP.

The optimal route of administration of glucocorticoids is controversial. A small double-blind randomized trial by Alam et al. (19), comparing IVMP and oral MP did not show a statistically significant difference at 5 and 28 days after treatment. The optimal preparation of glucocorticoids is also unclear. DX appears to be more beneficial than LDMP (16,27) but not than HDMP (16).

The role of pulsed HDMP as long-term treatment in RRMS is currently under investigation. Zivadinov et al. (28) have recently studied this issue in a randomized trial of 88 patients with RRMS. These patients were either pulsed IVMP (1000 mg/day for five days) followed by oral prednisone taper or the same dose of IVMP given only for relapses (28). Pulsed IVHDM was administered every four months for three years, then every six months for the next two years. Patients receiving pulsed IVHDM achieved a significant delay in progression of disability or cerebral atrophy. Fewer patients developed secondary progression, but there was, perhaps surprisingly, no effect on relapse rates.

High-dose corticosteroids have become the first-line treatment for acute relapses in MS. An yet unanswered question is whether the results of the ONTT are applicable to relapses of RRMS or clinically isolated syndromes other than ON. Furthermore, the optimal formulation, dose, route, and frequency of administration remain to be elucidated. Recent research has focused on role of long-term pulsed IVMP in delaying progression of disability and prevention of a secondary progressive course.

**INTRAVENOUS IMMUNOGLOBULIN**

IVIg treatment is effective for a number of immune-mediated demyelinating conditions such as Guillain–Barre syndrome or chronic inflammatory demyelinating polyneuropathy. The role of IVIg in treatment of MS is less clear. Most clinical trials have focused on long-term effects of IVIg in RRMS and secondary progressive MS. The role of IVIg for acute treatment of relapses remains to be elucidated.

IVIg has multiple mechanisms of action that may favorably influence autoimmune disorders, which has rendered challenging dissecting which of the proposed
mechanisms of action of IVIg treatment plays an important role in treatment of MS. Immunoglobulins can recognize and bind to the Fab region of antibodies; idiotype-antiidiotype networks play an important role in autoimmunity (29,30). Immunoglobulins can also bind complement components and prevent formation of membrane-attack complex. Changes in both CD8\(^+\) suppressor and cytotoxic cells and CD4\(^+\) helper T-cells have been demonstrated (30,31). Administration of IVIg can also modulate cytokine profiles in vivo (30) and mononuclear cells in vitro (32). Immunoglobulin preparations contain antibodies against interleukin (IL)-1 alpha, IL-6, and the class I and II interferons (30,33). Moreover, IVIg preparations contain trace amounts of anti-inflammatory cytokines such as transforming growth factor beta (30). Experimental evidence has also emerged for its role in remyelination. Rodriguez et al. (34) demonstrated that IVIg may have the potential to induce remyelination in the Theiler’s virus model of MS. A monoclonal IgM kappa antibody was identified, which recognizes antigens present on oligodendrocytes and other cells, promotes remyelination, and suppresses inflammation (30,35).

Van Engelen et al. (36) studied five patients with fixed visual deficits from ON and demonstrated improved visual acuity and color vision after one to two months of IVIg treatment. In another trial, Noseworthy et al. (37) performed a double-blind randomized trial in 55 patients with persistent loss of visual acuity after ON. Patients were randomized to receive either IVIg 0.4 g/kg daily for five days followed by 0.4 g/kg every four weeks for three months or placebo. There was no significant difference between the treatment groups in visual acuity at six months, although a trend favoring IVIg was found at 12 months. In another trial, Noseworthy et al. (38) studied the effect of IVIg treatments over three months in patients with stable neurological weakness, and found no beneficial effect of IVIg on relapse rate or impairment measures. Despite the experimental evidence for the role of IVIg in remyelination, there is no data for its role in restoration of function from stable neurological deficits. The data from the European intravenous immunoglobulin in secondary progressive multiple sclerosis (ESIMS) trial does not support the use of IVIg in secondary progressive MS (39).

Current evidence for the use of IVIg in acute relapses is sparse. Soukop and Tschabitscher (40) studied the use of IVIg (50 mg/kg) in 22 patients with an acute relapse and found clinical improvement in 15 patients (68%) within 24 hours; however, the benefit persisted for only two weeks. Sahlas et al. (41) reported dramatic clinical improvement in two patients with acute disseminated encephalomyelitis. Using serial gadolinium enhanced MRI, Nos et al. (42) studied the blood–brain barrier in patients with acute relapse receiving IVIg. This study compared IVIg treatment with a combination of IVIg and prednisone, and found a dramatic decrease in enhancement in serial scans in the latter group only. This argues against sealing of the blood–brain barrier being an important mechanism of action for IVIg.

Most clinical trials focused on the role of long-term IVIg treatment in RRMS, and there is limited data on use of IVIg in acute relapses. A series of IVIg treatments over a two-year time period may reduce relapse rate (43,44). Although this suggests that IVIg may favorably modify the course of MS, the clinical trials lack conclusive MRI data. Furthermore, available MRI results of the ESIMS trial do not support the use of IVIg in secondary progressive MS (45). Despite the lack of conclusive evidence for use of IVIg in the setting of RRMS, some trials have shown a clinical benefit and the agent deserves further attention, although other agents such as interferon beta and glatiramer acetate are generally considered better established and more convenient for the setting in which IVIg has been shown to be effective. Evidence that IVIg is
an effective “acute treatment” is purely anecdotal, and by analogy with its effectiveness of IVIg and TPE (see next section) in other autoimmune diseases such as Guillain-Barre syndrome and chronic inflammatory demyelinating polyneuropathy.

**THERAPEUTIC PLASMA EXCHANGE**

High-dose corticosteroids are the treatment of choice for acute demyelinating attack given their well-proven efficacy, as described above, and their ease of administration and cost. TPE may be a valuable treatment for patients who fail to respond to steroids. This section focuses on the use of TPE in acute attacks of demyelinating disease.

TPE has been studied as a potential treatment for patients with progressive form of MS and those with RRMS since 1980. This was first observed in a small number of patients with acute catastrophic attacks (46). Subsequently, a double-blind, randomized controlled clinical trial showed that TPE did not provide much, if any benefit, as an adjunct to ACTH and CTX for acute attacks of MS (47). In this study, the control group received sham TPE, and both groups received identical treatment with IM ACTH and oral CTX. Improvement in the treatment group receiving TPE was somewhat better at two weeks relative to the sham group, but the overall difference between the two groups over the period of observation was not significant. However, a trend of improvement at four weeks was noted in patients with RRMS treated with TPE, but not at 12 months. This study provided equivocal support that TPE may be beneficial in conjunction with ACTH and CTX, but TPE appeared to offer no long-term benefits. Different from the original observations that TPE may be effective for selected patients with catastrophic attacks, this study included patients with attacks of varying degrees of severity and those with progressive forms of MS. Another limitation of the study was the utilization of disability status scale (DSS) as primary end point, which may be insensitive to improvements in cognitive function or upper extremity dysfunction.

Shortly after this publication, Rodriguez et al. (48) reported six patients with acute fulminant episodes of CNS inflammatory demyelination who responded to therapeutic plasmapheresis after failing a course of IVHDMMP. All patients achieved dramatic improvement in motor and language functions within 2 to 14 days, and the therapeutic effect persisted during 6 to 35 months of follow-up. These results suggested that TPE might be valuable in treatment of severe episode of inflammatory demyelination in the absence of concurrent immunosuppressive treatment.

A number of uncontrolled clinical series reported on the effectiveness of TPE in acute inflammatory demyelinating disease (1,46). Weinshenker et al. (49) reported a randomized, sham-controlled, double-masked clinical trial of TPE without concomitant immunosuppressive treatment in patients with acute, severe inflammatory demyelinating attacks who failed to respond to corticosteroid treatment. These investigators selected 22 patients with severe neurological deficits unresponsive to steroid therapy. They included both patients with MS (n = 12), as well as patients with “atypical” demyelinating syndromes (n = 10), including acute disseminated encephalomyelitis, acute transverse myelitis, Devic’s neuromyelitis optica, and focal demyelination with mass effect. Functionally important (moderate to marked) improvement in the “targeted neurological deficits” without development of new neurological deficits or worsening of coexisting deficits was the primary endpoint. EDSS was felt to be insensitive to certain neurological
deficits such as cognitive dysfunction and aphasia. Patients who did not achieve significant improvement after the first treatment phase crossed over to the opposite treatment. This design provided access to the active treatment to all patients and increased the power of the study.

Eight of nineteen courses of active treatment (42%) resulted in moderate to marked improvement, as compared with one of seventeen (6%) courses of sham treatment. Furthermore, three patients who failed to respond to sham treatment experienced functionally important improvement in the second treatment phase after cross-over to active treatment.

Patients were followed for six months to determine if the response was sustained, although long-term benefit was not the primary endpoint of the study. Four of the eight patients who responded to the active treatment experienced new attacks during six months of follow-up. The remaining four patients subsequently did not experience another relapse for as long as four years of follow-up.

These results suggest that TPE may be valuable in selected patients with severe attacks of idiopathic demyelinating disease who fail to respond to steroids. On the basis of these results, TPE is now recognized as category II indication (supportive role) for acute treatment of demyelinating diseases by the American Society for Apheresis (2).

In a retrospective analysis of all patients treated with severe attacks of acute inflammatory demyelinating disease at Mayo Clinic from 1984 to 2000, Keegan et al. (50) confirmed moderate or marked functional improvement in 44.1% of patients. A somewhat higher response rate was observed in men, in those with preserved reflexes and treated within three weeks of the onset of their neurological deficit. The highest success rate (60% moderately or markedly improved) occurred in 10 patients with acute attacks of neuromyelitis optica, although the difference in the response rate in this subgroup was not significantly different than in those with prototypic MS. The overall response rate in this uncontrolled experience was comparable to that observed in the randomized prospective trial by Weinshenker et al. (49) which demonstrated efficacy of treatment. A recent report by the same group suggests that individuals who have type II pathology by the classification of Lassmann and Lucchinetti (oligodendrocyte precursors preserved with potential of remyelination; antibody and terminal complement membrane attack complex demonstrable by immunostaining) consistently respond to TPE, whereas those with other relatively common patterns without these features do not (51).

Mao-Draayer et al. (52) reported dramatic improvement in a patient with biopsy-proven tumefactive demyelinating lesion treated with TPE one week after failing to respond to IVMP, although spontaneous improvement or synergistic effect of steroids cannot be excluded. In a recent uncontrolled, retrospective observational study, Meca-Lallana et al. (53) studied the utility of TPE in 11 patients presenting with an acute attack unresponsive to intravenous MP. This group found significant clinical improvement during the first month of treatment in seven patients (77.7%). Ruprecht et al. (54) completed an uncontrolled observational study of 10 patients treated with TPE for acute, severe ON unresponsive to IVHDM. This group demonstrated improvement of visual function in 7 of the 10 patients studied. Spontaneous recovery cannot be completely excluded, as the study was uncontrolled unlike the Mayo Clinic randomized study; however, as in the Mayo Clinic study, the investigators followed the same paradigm by selecting only patients with the most severe deficits who were unresponsive to corticosteroid therapy. The authors argue that both the close temporal relationship of the observed clinical improvement with TPE and
the adequate length of time to observe recovery with the use of steroids before TPE argue against spontaneous recovery of visual function.

TPE may be valuable for treatment of acute severe inflammatory demyelinating relapses in patients unresponsive to corticosteroids. The preferred regimen, based on the controlled clinical trial that demonstrated efficacy, is a course of seven treatments administered every other day over a period of two weeks. The current literature provides strong evidence from a single randomized study, supported by uncontrolled studies, that patients with severe attacks unresponsive to conventional agents are likely to experience benefit from TPE. However, further investigations are required to noninvasively determine the subgroup of patients most likely to respond.

MITOXANTRONE

Several class II and III studies suggest a role for MTX in treatment of worsening RRMS or secondary progressive MS. MTX has been approved for treatment of worsening RRMS, secondary progressive MS, and progressive relapsing MS. MTX is an antineoplastic agent that has been used for prostate cancer and nonlymphocytic leukemia in adults. MTX produces DNA cross-links and strand breaks, interfering with DNA repair and RNA synthesis, thereby interfering with proliferation of and inducing apoptosis in lymphocytes (55,56).

Early experience with experimental transplantation suggested prolongation of survival of heterotopic cardiac transplants with the use of MTX (57). This agent was subsequently used successfully in the treatment of both actively induced and passively transferred experimental allergic encephalomyelitis (58,59).

In 1997, Edan et al. (60) reported the results of the French and British multicenter, randomized, nonblinded controlled trial of MTX in 42 patients with active CDMS treated with MP and MTX. Patients who entered the trial had either RRMS or secondary progressive multiple sclerosis (SPMS) and either two relapses with sequelae within the 12 months preceding entry to the study or progression of two points on the EDSS during the same time period. Three monthly gadolinium-enhanced MRI scans were performed in a baseline period of two months, and only patients developing at least one active MRI lesion during the baseline period were included. Patients were randomized to receive either monthly MTX (20 mg IV) and MP or MP alone over six months. A blinded analysis of MRI data showed a significantly greater number of patients in the MTX group without enhancing lesions than the control group (class II data). Furthermore, the clinical relapse rate was reduced and nonblinded clinical assessment showed a benefit for the MTX group (class III data). Fewer relapses were observed in the MTX group (7 vs. 31 relapses), and the difference was more pronounced during the last four months of treatment. Additionally, the MTX group experienced an improvement in the mean EDSS throughout the six-month period of observation (significant only in month 4), while the control group receiving only MP experienced sustained deterioration for up to four months. The sustained improvement in existing disability was somewhat unexpected, and may be consistent with the patients enrolled in this trial having an important reversible component to their disease, in essence an overlap between “relapse” and “progression.” On the other hand, the dramatic reversal of existing neurological deficits observed, which previously has not been documented with immunosuppressant therapy, may be explained by lack of blinding for clinical outcomes and subjective nature of the EDSS (56).
A phase IV multicenter, open-label study called Registry to Evaluate Novantrontrone Effects in Worsening Multiple Sclerosis is being conducted to obtain information on long-term effects of MTX in patients with MS.

Identification of the appropriate patient population that would best respond to MTX is of utmost importance when considering this treatment in face of potential serious toxicity. This drug is optimally used in the setting of rapidly worsening MS refractory to treatment with steroids, and should be considered in patients with MS with significant clinical deterioration refractory to other therapies. Nausea, alopecia, bone marrow dysfunction, and gonadal dysfunction including amenorrhea and cardiotoxicity are potential adverse effects. The usual dose used by the authors is 12 mg/m² given at three monthly intervals until a maximum cumulative dose of 140 mg/m² is reached. The clinical response should guide the length of treatment. Baseline hemoglobin level, white blood cell count (including differential), and platelets should be obtained in all patients receiving MTX, approximately three to five days prior to the treatment, and should be repeated prior to subsequent infusions. Leukopenia is generally expected to recover within the first three weeks of the therapy. Patients should have a baseline cardiac assessment and repeat cardiac assessment including a measure of the ejection fraction, when a cumulative dose of 100 mg/m² is reached. A significant drop in ejection fraction or an ejection fraction of less than 50% precludes further therapy. Significant cardiac risk factors, known heart disease, or prior history of mediastinal radiotherapy are recognized as contraindications. Liver and kidney functions should also be monitored during therapy but are not commonly altered by treatment.

**CYCLOPHOSPHAMIDE**

CTX is an alkylating agent with immunosuppressive properties and is commonly used in treatment of immune-mediated disease. The role of CTX in MS has been studied extensively, and the current literature supports its use in active inflammatory demyelination. Uncontrolled data suggests that CTX may be an effective alternative for treatment of rapidly worsening MS. However, it appears to be ineffective in most cases of slowly and gradually worsening progressive MS.

In an open-label, nonblinded, uncontrolled study, Weinstock-Guttman et al. (61) treated 17 consecutive patients with fulminant MS, refractory to corticosteroid treatment, with IVCTX 500 mg/m² with IVMP 1.0 g for five consecutive days, followed by a five-day tapering course of prednisone. Maintenance immunotherapy was initiated about eight weeks after CTX/MP induction, and consisted of methotrexate, MP, or interferon beta-1b at the discretion of the treating neurologist. Patients were followed for 24 months. Thirteen of seventeen (76%) and ten of seventeen (59%) patients improved after three and six months, respectively. Thirteen of seventeen (76%) patients were stable or improved after one year and nine of thirteen (69%) after two years. All patients who worsened after three months continued to deteriorate during this follow-up period despite maintenance immunotherapy. Of 10 patients who were nonambulatory at the time of induction therapy (EDSS ≥ 8.0), five (50%) became ambulatory. The authors suggested that CTX/MP may represent an effective therapeutic option for the rare MS patients with a fulminant progressive course.

Khan et al. (62) treated 14 consecutive CDMS patients who had a clinical course marked by severe deterioration refractory to conventional immunomodulatory
agents and IVMP in the preceding year with CTX. All patients stabilized or improved at six months, and the benefit was sustained at 18 months after the onset of treatment with CTX. In a recent unblinded, uncontrolled study, Patti et al. (63) studied the effects of combined treatment with CTX and interferon-beta in selected patients with "rapidly transitional" MS who were previously treated with beta interferon. Monthly treatment with CTX administered to produce a lymphopenia of 600 to 900/mm$^3$ produced a significant reduction in the relapse rate, disability, and reduction of T2 MRI burden of the lesion as compared with the beta interferon treatment period preceding the study. The treatment was safe and well-tolerated in the short term follow-up of this study. Potential side effects include nausea, vomiting, bone marrow suppression with leukopenia, transient alopecia, amenorrhea, oligospermia and infertility, bladder toxicity, and potential for bladder and hematological malignancies.

Despite its controversial role in progressive disease, CTX may be effective in selected patients experiencing rapid progression of disability refractory to conventional therapy, similar to the situations in which MTX is appropriately administered. These immunosuppressant drugs are most effective when administered in active inflammatory disease to arrest rapidly deteriorating neurological dysfunction. With the approval of MTX for rapidly worsening MS, many clinicians have used this treatment in lieu of CTX, although cardiotoxicity is not problematic for CTX. There is no direct comparative study of CTX and MTX.

CONCLUSIONS

Acute treatment in demyelinating conditions is usually considered for either relapses or rapidly worsening MS (Fig. 1) to reverse neurological disability, arrest neurological deterioration, and restore function. An algorithm, illustrating our suggested approach to acute treatment, is given in Figure 2. "Pseudoexacerbations" are transient symptomatic deterioration of neurological function which may occur in the setting of an underlying infection, which must be promptly recognized and treated, rather than instituting corticosteroid or immunosuppressive treatments. Disabling relapses should be treated with short courses of corticosteroids, usually given as IVHDM 1.0 g for three to five days, which may or may not be followed by one to two weeks of oral prednisone on a tapering schedule. This approach is believed to shorten the course of an attack but has not been shown to alter the ultimate outcome and disability. The optimal corticosteroid preparation, dose, route, and frequency for treatment of acute relapses remain to be clarified. However, the results of the ONTT, La Mantia et al. (16) and the earlier reports suggest that the HDMP is superior to the other formulations (Table 1).

A small proportion of patients refractory to conventional treatment with steroids may develop a rapid deterioration and severe worsening of their disability. "Catastrophic MS" can generally be classified into one of two patterns: (i) acute fulminant or (ii) rapidly worsening inflammatory demyelinating disease (Fig. 1). It is important to exclude other disorders that may mimic such severe inflammatory demyelinating attacks and deterioration, particularly for patients who are experiencing a first such event or those for whom a diagnosis of MS is as yet uncertain. Patients experiencing an acute fulminant attack unresponsive to corticosteroids should be treated with TPE, which may be given as seven treatments, approximately every other day. A number of uncontrolled clinical series and the randomized, controlled trial by Weinshenker et al. (49) reported in 1999 suggest a role for TPE in
treatment of patients with severe demyelinating attacks refractory to steroids. The American Society for Apheresis has recognized TPE as category II indication for treatment of acute demyelination based on that study. The role of IVIG as an alternative rescue therapy remains to be elucidated.

Patients who present with a step-wise worsening associated with frequent relapses or with rapid but continuous progression of disability associated with MRI evidence of inflammatory demyelinating disease (i.e., new lesions or enhancing lesions) may be reasonable candidates for immunosuppressive therapy. Alternative, nondemyelinating conditions should be excluded. MTX or CTX are the most widely used contemporary treatments to achieve rapid suppression of disease activity in those who have clinical or radiographic signs of active inflammation associated with rapid clinical deterioration. If immunosuppressive agents are successful, maintenance therapy with interferon beta should be considered after an initial course of treatment with MTX or CTX. Other immunosuppressive agents or long-term IV HDMP pulse are administered occasionally in this setting. Finally, some groups are exploring autologous stem cell transplantation as an alternative means to achieve long lasting global immunosuppression. Guidelines have been published and phase I studies have been completed to determine the safety of the procedure (64). Early experience with uncontrolled pilot studies appears promising, but phase II and III trials are needed to determine clinical efficacy.

REFERENCES


Treatment of the Clinically Isolated Syndromes

Giancarlo Comi
Department of Neurology and Clinical Neurophysiology, Vita-Salute University, San Raffaele Scientific Institute, Milan, Italy

INTRODUCTION

Multiple sclerosis (MS) is a disease which produces in the vast majority of the patients significant disability (1,2). The available immunomodulatory treatments are not a cure for MS, but there is a clear evidence from class I clinical trials that they significantly reduce disease activity and delay the increase of disability in relapsing–remitting (RR) patients (3–10), while the positive effects are less clear in secondary progressive patients (11,12). The different effects of immunomodulatory treatments according to the disease course is probably explained by the complex pathogenesis of MS. Indications on the use of available therapies for MS have substantially changed over the last few years, from a conservative (13,14) to a more aggressive attitude (15). It is interesting to note that the consensus statement of the Canadian MS Clinic Network, recently published (15) on the use of disease modifying agents in MS, requires evidence of ongoing disease activity, which can be based on clinical or magnetic resonance imaging (MRI) data, while previous consensus of treatment (13,14,16,17) required two or more relapses in the last two years in order to start the treatment. These changes are probably explained by the results of new trials testing the efficacy and safety of interferons (IFNs) and glatiramer acetate (GA) (5–7,10), by the experience acquired during these years and by the recent investigation of the pathophysiology of the disease. The demonstration of early irreversible axonal damage is a strong argument in favor of early treatment, an option which is beginning to be shared by many neurologists (18,19). The McDonald diagnostic criteria recently validated (20,21) allows one to advance the decision to treat.

RATIONALE FOR EARLY TREATMENT

Many factors, summarized in Table 1, support the early treatment in MS. MS is a severe disease. About 80% of patients have a progressive course within 20 to 25 years from onset (1,22). Natural history studies in clinically isolated syndrome (CIS)
patients have many weaknesses: the depth of the clinical investigations performed at presentation, the percentage of patients lost to follow-up, the accuracy of follow-up evaluations etc. Clinical trials in CIS offer a more reliable collection of data, regular sampling, and a low proportion of patients lost to follow-up; however, the follow-up period is short. The frequency of CIS converting to clinical MS in two to three years duration clinical trials ranges from 16.7% in optic neuritis treatment trial (ONTT) to 45% in early treatment of multiple sclerosis (ETOMS) study. In general, patients with optic neuritis (ON) seem to have a lower risk for conversion to clinically definite multiple sclerosis (CDMS) than patients with another CIS, as revealed by the 10 years follow-up of the ONTT study (23). The frequency of conversion was 38% and most patients who developed CDMS had a relative benign course (24). Clinical trials performed in CIS failed to confirm that the anatomic site involved at presentation predicts the risk to conversion to CDMS. The discrepancies between epidemiological studies and clinical trials could be explained by the more strict inclusion criteria used in clinical trials to include patients with isolated visual disturbances. Some clinical and MRI findings, observed at clinical presentation of MS, are predictive of the evolution to CDMS and of future disability (Tables 2 and 3). The most important factors are the amount of nervous tissue affected by the disease and the presence of active brain lesions as revealed by MRI techniques (25–27). In the study performed by Filippi et al. (25) in patients with isolated syndromes, the baseline T2 lesion load was predictive of the future disability. This original observation was confirmed by subsequent studies. A group of 109 patients with CIS entered a long-term

<table>
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<th>Table 1</th>
<th>Rationale for Early Treatment of Multiple Sclerosis</th>
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<td>Disease severity</td>
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<td>Antigen spreading</td>
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<td>Early course influence long term evolution</td>
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<td>Longitudinal changes of immunopathology</td>
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<td>Irreversible nervous damage occurs very early and is (at least partially) related to inflammation</td>
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<td>Recovery mechanisms may become less effective during the course of MS</td>
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<td>Immunomodulating treatments affect immunomodulation which predominates in the early phases</td>
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<td>Positive results of CHAMPS and ETOMS</td>
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<td>Evidences of a better response to IFNβ in the early phases of the disease</td>
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*Abbreviations:* MS, multiple sclerosis; CHAMPS, Controlled High Risk Subjects Avonex Multiple Sclerosis Prevention Study; ETOMS, early treatment of multiple sclerosis; IFNβ, interferon-β.

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<th>Table 2</th>
<th>Clinical Prognostic Factors in Multiple Sclerosis</th>
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<td>Male sex</td>
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<td>Age at onset &gt;40 years</td>
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<td>Polysymptomatic presentation</td>
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<td>Involvement of cerebellar, pyramidal and sphincters FS</td>
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<td>&gt;5 relapses in the first 2 yrs</td>
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<td>Short interval between first and second attack</td>
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<td>Incomplete recovery from the first attack</td>
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<td>EDSS at year 5</td>
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*Abbreviations:* EDSS, expanded disability status scale; FS, functional system.
follow-up: 81 patients were followed for 10 years (26) and 71 patients for 41 years (28). The conversion to MS was associated with the presence of abnormalities in baseline MRI, and was independent from the overall severity of lesions. However, the baseline lesion number correlated with the long-term disability and there was a correlation between increase of lesion volume and increase of disability (the strength of correlation was higher in the first five years of follow-up). In the ONTT, the 10-year risk of MS following an initial episode of acute optic neuritis was significantly higher if brain MRI was positive, higher numbers of lesions did not appreciably increase the risk (23). In the ETOMS patients with nine or more T2 lesions in the brain MRI compared to patients with four to eight lesions had a more frequent conversion to clinically definite MS over two years (29). Interestingly enough the risk was the same in patients with 9 to 25 lesions and in patients with more than 25 lesions. In the same study, the presence of enhancing lesions at the baseline scan, which was performed in two-thirds of patients two to three months after the attack onset, suggests that the persistence of inflammatory activity is predictive of the future conversion to CDMS (29). Similar results were observed in the Controlled High Risk Subjects Avonex Multiple Sclerosis Prevention Study (CHAMPS) the two years cumulative probability to develop clinically definite MS in the placebo arm was 57% in patients with one or more enhancing lesions in the baseline MRI compared with 33% in patients without enhancing lesions. Very recently an elegant epidemiological study demonstrated that the longer the duration of MS and the lower the disability, the more a patient is likely to remain stable. The five years status (clinical and MRI) seems to be a good prognostic factor for the next 10 years course, as already suggested by other studies (30,31). There are at least two possible explanations for these early prognostic factors. Some genetic factors might influence the disease evolution, for instance some patients may accumulate lesions faster than others. This interpretation is supported by the heterogeneity of MS pathogenesis (32). Soderstrom et al. (33) in a follow-up study performed in ON patients found that the Dw2 phenotype was related to the development of MS. Sciacca et al. (34) found that a more aggressive disease course was associated with Al/Al genotype of the anti-inflammatory cytokine interleukin-1 receptor antagonist. A second possibility is that patients with more brain MRI lesions at onset have a longer subclinical phase of the disease; however, this interpretation does not explain why patients with a multifocal rather than unifocal presentation as indicated by clinical findings also more frequently convert to CDMS (29). Moreover, it is well known that a high relapse rate in the early phases of the disease and a short interval between first and second attack are related to a worse prognosis (35,36). If a high clinical and MRI activity in the early phases results in a more rapid accumulation of irreversible disability, we can expect that a treatment able to reduce disease activity in the early phases of the disease may substantially ameliorate the long-term prognosis.

Table 3  Prognostic Factors in Clinically Isolated Syndrome (ETOMS-CHAMPS)

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<th>Factor</th>
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<tr>
<td>≥9 T2 lesions</td>
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<td>&gt;1 Enhancing lesion</td>
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<tr>
<td>Multifocal presentation</td>
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<tr>
<td>Severe attack</td>
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*Abbreviations:* ETOMS, early treatment of multiple sclerosis; CHAMPS, Controlled High Risk Subjects Avonex Multiple Sclerosis Prevention Study.
Immunological Findings

About 90% of MS patients display an abnormal B-cell response in spinal fluid examination, as revealed by the presence of oligoclonal bands (OBs). The frequency of positivity is usually lower in CIS, being 83% in ETOMS. The presence of OBs increases the risk of conversion to MS (37); however, the analysis of covariance demonstrated that increased risk is mostly due to the presence of brain MRI abnormalities. Most of these (OBs) do not react to neural antigens, so their pathogenetic role is debated. It is known that antibodies against myelin antigens may be detected in early MS (38), but, they can also be observed in normal subjects. Antibodies against myelin oligodendrocyte glycoprotein (MOG) are of special interest because they cause demyelination in vitro (39) and in animal models of MS (40) and have been found in active lesions of MS patients (41). In a recent study performed in 103 patients with selective CIS (positive brain MRI and presence of OBs) followed for at least one year, the presence of serum anti-MOG was associated to an increased risk of early conversion to CDMS. The adjusted hazard ratio for the development of CDMS was 31.6 among the patients who were seropositive only for anti-MOG antibodies, when compared with the seronegative patients, the value increased to 76.5 among the patients who had concomitant seropositivity also for anti-myelin basic protein (anti-MBP). Interestingly enough, patients with both anti-MOG and anti-MBP antibodies had higher mean number of T2 and T1 enhancing lesions. Increased frequency of anti-MOG antibodies in CIS has been confirmed in another recent study (42), but with a lower frequency of positivity. On the contrary, using a liquid-phase radiobinding assay the increased frequency of anti-MOG antibodies in MS patients has not been confirmed (43) and another study performed in CIS did not find an association between the presence of anti-MOG antibodies and early conversion to MS (44). Methodological problems and differences in the patient population examined could explain the different results; further validation studies are needed.

To date there is no definite evidence that immunological abnormalities observed in early and late phases of the disease differ significantly. However, some scattered indications support the possibility of an increased complexity of the immunological derangement over time underlying the diverse pathogenesis of MS. In autoimmunity, regulatory cells tend to recognize more epitopes within the same antigen, and more antigens within the same organ over time during the progression of the disease; this process which is called inter-/intraepitope spreading has been shown to be a feature of CNS antigen-specific T-cells in animals with experimental allergic encephalomyelitis (EAE) (45). Mice immunized with the immunodominant proteolipid protein (PLP) 139–151 determinant had an intra- and intermolecular sequential determinant spreading. Interestingly enough, mice only with relapsing progressive courses had the spreading of recognition to new immunodominant encephalitogenic determinants (45). An amplification of the autoimmune process has also been demonstrated in MS patients and could account for disease progression (46,47). However, it is not clear whether autoreactivity stabilizes with time or maintains a high level of diversity and plasticity during the disease.

Patients with progressive MS show significantly increased interferon gamma (IFN-\(\gamma\)) production compared to relapsing–remitting multiple sclerosis (RRMS) patients when T-cells are stimulated with anti-CD3 antibody. This increased production is IL-12 dependent and progressive MS patients show increased IL-12 production compared with RRMS patients (48,49). These data suggest that the
inflammatory process may have different characteristics as the disease evolves; if these phenomena play a role in the disease evolution, an early immunomodulatory treatment leading to downregulation of antigen-specific T-cells and the selective activation of specific cytokine networks could give better outcomes than delayed treatment and slow disease progression.

Pathology and Pathophysiology

There are many converging clinical, pathological, neurophysiological, and MRI lines of evidence that irreversible axonal damage occurs in the early phases of the disease, even if the degree of damage can be quite variable from patient to patient.

Pathological observations might explain the interpatients variability of MS course. There are multiple pathological patterns in MS, probably subtended by variable pathogenetic mechanisms (50). However, in the same patient, at a given time, all the lesions share the same pathological patterns (51). Whether the same pathological pattern will persist throughout the entire life of the patient is still unclear; if this is the case, then the prognosis could be determined in the early phases of the disease and specific therapeutic strategies could be consequently adopted.

The pathological substrates of symptoms and signs in MS are demyelination and axonal degeneration. Demyelination results in an instability of nerve conduction and generation of ectopic impulses, responsible for some typical positive symptoms of MS, such as Lhermitte sign, and negative symptoms and signs due to conduction block. Conduction block is due to segmental demyelination and concurrently by the action of toxic substances, such as nitric oxide and free radicals, produced by the inflammatory reactions, which has easy access to axons exposed by demyelination (52).

Reversible conduction block is responsible for the transitory neurological dysfunction observed in acute bouts. It is still debated if a persistent conduction block may also arise in the CNS, as has been demonstrated in the peripheral nervous system, for example in the multifocal motor conduction block neuropathy. Nevertheless, it is clear that the pathological basis for persistent neurological dysfunction in MS is axonal damage.

In Charcot’s description in 1877 (53), the axonal pathology inside MS plaques was considered of limited importance. This view was accepted for a long time. Putman in 1936 (54), first claimed the importance of axonal pathology in MS: in a postmortem study, he found a severe axonal loss in 50% of plaques. To the contrary, Grenfield and King (55) in the same year reported a nearly normal axonal density in most plaques. Some recent pathological studies have contributed substantially to demonstrate that axonal pathology occurs also in the early phases of the disease. The pathophysiology of axonal damage is quite complex and not fully understood. At least two different mechanisms could be hypothesized.

Early Axonal Damage

Ferguson et al. (56), using β-amyloid precursor protein, demonstrated the presence of damaged axons in both the acute and active chronic MS lesions, i.e., in areas of acute inflammation. Trapp et al. (57), in a very elegant study, utilized confocal microscopy and immunohistochemistry to demonstrate a large number of transected axons in active lesions. The frequency of terminal axonal ovoids, indicating recent
Axonal transection, correlated with active inflammation. Axonal damage might be a consequence of the loss of myelin exposing the axon to the products of inflammation or a humoral immune response could perhaps contribute to irreversible axonal damage. Raine et al. (58) very recently provided evidence that the antibody to MOG may contribute to the myelin damage. Antibodies to MOG have been shown to be specifically bound to disintegrated myelin around axons in acute MS lesions as well as in marmoset EAE. Moreover, we cannot exclude the pathogenetic role of antibodies to axonal components.

Axonal loss is predominant in lesions appearing in the early phases of the disease (59) and decreases over time. A high amount of damage occurs in areas with large infiltration of T-lymphocytes (especially CD8+ T-cells) and macrophages indicating a correlation between inflammation and axonal damage (59).

Because of redundancy in the organization of the CNS and because of the convergency/divergency of the multisynaptic pathways the initial axonal loss does not produce permanent symptoms and signs. However, new lesions affecting the same pathways or reactivation of old lesions will result in a severe axonal loss and will lead to irreversible neurological dysfunctions.

Magnetic resonance spectroscopy (MRS) (60–64), magnetization transfer imaging (MTI) (65–68), brain and spinal cord atrophy measures (69–72), and T1 black holes (73) provide indirect evidence of axonal loss as an early phenomenon in MS. There is a correlation between axonal loss and magnetic transfer ratio (MTR) both in plaques (74) and in normal appearing white matter (NAWM) (75). The diffusion-weighted imaging, the MRS and the magnetization transfer clearly demonstrate that the NAWM also affected in MS, as a result of the axonal degeneration and probably also because of small foci of inflammatory activity, undetected by conventional MRI techniques (62,76,77). In CIS patients with clinical symptoms related to motor function, the DT-derived mean diffusivity and lesion volume in the pyramidal tract were found to be increased (78). Magnetization transfer histograms of the normal appearing brain tissue in patients with isolated syndromes revealed subtle changes outside visible lesions, the severity of which was predictive of future development of CDMS (79). Ventricular enlargement is also present in these patients and predicts the future development of CDMS (80). Of great interest is the observation, in a post hoc analyses of two subgroups of patients participating in the IFNβ-1a (Avonex, Biogen) trial in RRMS, that during the second year the brain atrophy progressed significantly less in the treated group then in the placebo group (81). A group of untreated patients, in the same trial, underwent corpus callosum and third and lateral ventricles width measures; corpus callosum atrophy and ventricular dilatation significantly increased during the two year follow-up (82). The increase of ventricular width was associated with increase of disability and was predicted by the baseline number of gadolinium enhancing lesions. In the ETOMS study, there was a significant correlation between number of active lesions and progression of brain atrophy: treatment with IFNβ-1a reduced the progression of brain atrophy by one-third (83). In a 18-month follow-up study performed in 62 CIS patients, brain MRI activity significantly correlated to the progression of brain atrophy (84). However, inflammation and brain atrophy did not proceed in parallel: atrophy appeared only after a delay of months following acute inflammation.

Functional MRI studies provide evidences of an early cortical adaptation to nervous damage in MS which may contribute to early recovery (85–87). The extension of the cortical reorganization is a marker of the severity of early tissue loss and could have prognostic implications.
Secondary Axonal Degeneration

Trapp et al. (57) found also diffuse abnormalities in surviving axons, discontinuous staining of the axons and modifications of the axonal caliber, which could explain a shortening of the axonal life leading to a subsequent secondary degeneration. In fact, the authors describe also the presence of terminal axonal ovoids in the hypocellular center of chronic active lesions. This finding cannot be explained by a direct inflammatory insult, but can be related to a continuous “degenerative” process which could be the substrate of the continuous progression characterizing the intermediate and advanced phases of the disease. The clinical observation that in the progressive phase of the disease, the spatial distribution of sensory-motor deficits has the classical distal–proximal gradient strongly support the important role of secondary degenerative processes. This process becomes clinically evident only when the safety factor (number of functioning axons) is joined. Very recently, Lovas et al. (88) performed a postmortem study of the cervical spinal cord in a group of patients with secondary progressive MS. They found that axonal density was reduced both in the plaque and in the NAWM; at least two-thirds of the axons were lost in inactive, chronic lesions. Moreover, axons were thinner in plaques than in the NAWM. The authors concluded that their observations support the concept of slow axonal degeneration rather than acute damage as a cause of chronic disability. Similar findings have also been found by Trapp (personal communication) and are consistent with the atrophy of the cervical spinal cord demonstrated in these patients by MRI (69,70). The amount of this secondary degeneration compared to the acute degeneration is unknown. Many interpretations have been proposed to explain the secondary degeneration. Naked, demyelinated axons may be more susceptible to degeneration because they lost the trophic support from the oligodendrocyte, a hypothesis supported by the observation that remyelinated axons are protected from further damage (59). During the early RR phase of the disease, extensive remyelination is usually observed, which explains the complete recovery characterizing most of the bouts (89,90). Remyelination depends upon the availability of oligodendrocytes or their progenitor cells within the lesions (91,92). It has been suggested that, the failure of myelin repair in late chronic lesions could be due to a depletion of this progenitor cell pool, which is likely to occur in areas of repeated demyelinating episodes (93). Both pathological studies and MRI studies revealed that about 30% of the active lesions are old reactivated lesions, the so called shadow plaques. The same findings have been clearly demonstrated in EAE. A primary pathology of oligodendrocytes could also explain the inefficient remyelination in some cases, an explanation which has been proposed for patients with primary progressive MS (94). Finally, we should consider the extreme hypothesis that MS is a primary progressive degenerative disease, with a secondary inflammatory response.

Clinical Trials

The first evidence of the potential positive effects of an early anti-inflammatory treatment in MS derives from the ONTT. The trial demonstrated that a single course of three days of 1g of intravenous methylprednisolone reduced the risk by about 50% at two years of conversion to clinically definite MS (95), on the contrary, oral steroid treatment had no effect. The beneficial effect of high dose steroids was transitory, being lost at the five years follow-up (96) and could be explained by the acute anti-inflammatory effect of steroids.
There are some indications that the effects of IFNβ-1a (Rebif, Serono) on disease activity may vary with the disease phase. In the prevention of relapses and disability by IFNβ-1a subcutaneously in multiple sclerosis (PRISMS) study, patients with an EDSS score ≤ 3.5 at entry and the high dose of IFNβ-1a reduced the clinical and MRI activity to the same extent, while in patients with an EDSS score > 3.5 the proportion of patients free from exacerbation and with inactive scans was significantly reduced in the high dose only (6). These data suggest that patients in the early phases of the disease could benefit lower doses of IFNβ-1a. In the same way the trial testing GA in RRMS (9) showed that the therapeutic effect appeared to be most pronounced in patients with the lowest EDSS score at entry.

Comparison of results across clinical trials must be interpreted with caution because the observed differences could be explained by interactions of many factors. Nevertheless, it is important to note that the same dose of IFN given to patients with CIS and RRMS has quite different results: 22 mg of IFNβ-1a (Rebif) given once a week subcutaneously had no effects in RRMS patients and significantly reduced the disease activity in CIS. The proportion of patients free from exacerbation and the time to the first exacerbation were significantly increased in the Avonex group compared with the placebo group in the CHAMPS study. On the contrary, the two parameters were not significantly modified in the pivotal North American trial in RRMS testing the efficacy of the same dose of Avonex.

Brain atrophy is a good and reproducible measure of the accumulation of irreversible tissue loss. As it is already evident in the early phases of the disease, the precision of this measure makes it possible to detect changes also in follow-up of short duration. The effects of treatments on brain atrophy have been tested in many clinical trials and the results are summarized in Table 2. In patients with CIS treated with Rebif, the progression of brain atrophy during the two years follow up was significantly reduced compared to patients receiving placebo (97). Interestingly enough, the progression of brain atrophy was correlated to the number of active lesions accumulated during the follow-up. In the North American Avonex trial in RRMS the progression of brain atrophy was significantly reduced in the second year of treatment in the actively treated group compared with the placebo group (98). Sormani et al. (99) in a recent paper investigated if GA had a beneficial effect on the development of brain atrophy in the two groups of RRMS patients of the European/Canadian Multicenter, Double Blind, Randomized, Placebo Controlled Study on MRI-monitored disease activity. The reduction in brain volume in the first phase of the study was 0.8% and 0.9% in GA-treated and in placebo patients, respectively. In the second phase brain volume continued to decrease, however, by only 0.6% for patients always on GA and 1% for those originally on placebo, a difference statistically significant. Interestingly enough, all clinical trials performed in secondary progressive MS failed to show a significant effect of IFNs on brain atrophy. In conclusion early treatment reduces the progression of brain atrophy, while no effects were observed in patients in the secondary progressive phase of the disease.

**ETOMS-CHAMPS**

The results of two recent double-blind placebo-controlled clinical trials [ETOMS (29) and CHAMPS (100)] are supportive of early treatment of MS.

The European ETOMS trial enrolled 308 patients with onset of a first monosymptomatic or polysymptomatic syndromes suggestive of MS no more than three months before study entry and with a brain MRI suggestive of MS. The patients were
randomized to receive 22 µg of IFNβ-1a (Rebif, Serono) by subcutaneous injection once a week or placebo for two years. The proportion of patients converting to clinically defined MS (CDMS) was significantly lower for the IFNβ-treated group than for the placebo group (34% vs. 45%, \( P = 0.047 \)) with a 24% relative reduction of conversion risk with the active treatment. The time at which 30% of patients had converted to CDMS (occurrence of a second relapse) was 569 days in the IFNβ group and 252 in the placebo group (\( P = 0.034 \)). The annual relapse rate was lower in the IFNβ group (0.33) compared with the placebo group (0.43) with a reduction of 23%. There were significantly fewer new T2 lesions in the IFNβ-1a group than in the placebo group (\( P < 0.001 \)). The proportion of patients without MRI activity during the study was significantly higher in the IFNβ group than in placebo group (16% vs. 6%, \( P = 0.005 \)). At the end of the study, there was an increase in T2 lesion volume of 8.8% in the placebo group compared with the baseline value while in the IFNβ group there was a decrease of 13% (29). Of the original 154 patients of the placebo arm, 129 entered the extension phase and 120 completed it. Of the 154 patients randomized to Rebif, 134 entered the extension phase and 115 were reexamined after a mean followup of 4.4 years. During the extension phase, all patients received Rebif 22 mg once a week. At the last visit, the proportion of patients converted to MS were 57.8 in the placebo arm and 46.1 in the Rebif arm (\( P = 0.05 \)) (Fig. 1). There was a trend in favor of Rebif for the increased time to conversion and for the proportion of patients free from confirmed increase of disability (17.5% vs. 22.7%). The results of the extension study suggest that the early treatment with a very low dose of IFNβ-1a continue to produce some benefits compared to a delayed treatment.

The American CHAMPS trial enrolled 383 patients with onset of a first single monosymptomatic syndrome suggestive of MS no more than two weeks before study entry and with a brain MRI suggestive of MS. The patients were randomized to

![Figure 1](image_url) Time to conversion to clinically definite multiple sclerosis.
receive 30 μg of IFNβ-1a (Avonex<sup>®</sup>, Biogen), by intramuscular injection once a week or placebo. The proportion of patients converting to CDMS was significantly lower for the IFNβ treated group than for the placebo group (35% vs. 50%, \( P = 0.002 \)). When compared with the patients in the placebo group, patients in the IFNβ-1a group had a relative reduction in the volume of brain lesions on T2-weighted MRI scans (\( P < 0.001 \)), fewer new or enlarging lesions on T2-weighted MRI scans (\( P < 0.001 \)), and fewer gadolinium-enhancing lesions on T1-weighted scans (\( P < 0.001 \)) at 18 months (100). A beneficial effect of treatment was noted in all subgroups. Adjusted rate ratios for the development of CDMS in the optic neuritis, brainstem-cerebellum, and spinal cord syndrome subgroups were: 0.58, 0.40, and 0.30. Treatment benefit was observed regardless of age, gender, race, duration of pretreatment period, and baseline brain MRI characteristics. A beneficial effect of the trial over a five years period demonstrated that early treatment with Avonex reduced the probability of developing MS by 35% compared to delayed treatment.

The effect of IFNβ-1a treatment was slightly greater in CHAMPS than in ETOMS and may be related to differences in the dose administered (30 μg in CHAMPS vs. 22 μg in ETOMS) and in the different inclusion criteria. The CHAMPS trial included monosymptomatic patients, whereas the ETOMS study included both monosymptomatic and polysymptomatic patients and the risk of conversion was about two times higher for multifocal than unifocal presentation in ETOMS study; moreover, the delay between the onset of the first attack and inclusion in the trial was shorter in the CHAMPS study (two weeks) than in the ETOMS study (three months) and this difference could lead to subtly different populations. The median T2 lesion volume at the baseline was higher in ETOMS than in the CHAMPS study suggesting a more severe group in ETOMS.

The extension phase of the two studies produced very similar results indicating the importance of the anticipation of the treatment. It is very important to note that two independent studies reached the same conclusions. Moreover, the two studies produced very useful indication on patients at high risk of an early reactivation of the disease (L) discussed in detail earlier. These prognostic factors can be used to select CIS candidates to an immediate or a delayed treatment.

**Intravenous Immunoglobulins**

Intravenously administered immunoglobulins (IVIg) treatment has been reported to be beneficial in the treatment of patients with RRMS (101,102). IVIg has been recently studied in a placebo controlled trial in 91 patients enrolled within the first six weeks of neurological symptoms (103). The cumulative probability of developing CDMS was significantly lower in the IVIg treatment group compared with the placebo group (rate ratio 0.36, \( P = 0.03 \)). Number and volume of T2-weighted lesions and volume of the T1-enhancing lesions were also significantly reduced in the IVIg group compared to placebo group. The short duration of the follow-up (one year) and the small size of the study limit the interpretation.

**Ongoing Clinical Trials**

The recent demonstration that multi-weekly injections of IFNβ is significantly superior to weekly injections in patients with RRMS (104) is now being studied in patients with CIS. The efficacy of IFNβ 1b s.c. every other day will be tested in a double-blind placebo-controlled trial lasting two years. In addition, patients will enter an extension phase of three years to compare immediate and delayed IFNβ treatment on disease
activity and disability progression. A clinical trial of GA is also in progress in patients with CISs and unifocal presentation.

CONCLUSION

The aforementioned clinical, immunopathological, and imaging data suggest that the early treatment of MS patients with immunomodulatory drugs is advantageous compared with treatment started later in the disease course. Since disability accumulated in the first five years after onset corresponds roughly to three-fourth of the disability status after 15 years, the early reduction of relapse rate as well as of the extent of pathological lesions should be the strategy for patients. Early treatment has a robust rationale both in preventing irreversible changes and in reducing clinical and MRI activities with favorable prognostic implications.

All patients with a diagnosis of RRMS, who are in an active phase of the disease are candidates for treatment. In CIS patients a treatment option should be considered in presence of negative prognostic factors for an early reactivation of the disease (Table 3). The key point in CIS is the extensive exclusion of other possible diseases, including cerebrospinal fluid examination and the careful evaluation of MRI findings. The number, morphology, and location of the lesions are very important contributors to the diagnosis. If there are doubts about the diagnosis it would be better to delay treatment, even in presence of clinical and instrumental negative prognostic factors! There are still some concerns about the long-term advantages of the early treatment of MS. Detractors of this strategy claim that there are no proofs that long term disability is influenced by the positive effects of immunomodulatory treatment on disease activity. Ongoing clinical trials in CIS will hopefully contribute to solve these objections.

REFERENCES

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INTRODUCTION

The cause of multiple sclerosis (MS) is unknown although there is a large body of experimental evidence to suggest that activated T-cells, reactive to self-antigens such as myelin basic protein, myelin oligodendrocyte glycoprotein, myelin associated glycoprotein, or proteolipid protein proliferate, and under the influence of cellular adhesion molecules and pro-inflammatory cytokines, cross the blood–brain barrier and enter the central nervous system (CNS) to produce the inflammatory lesions seen in MS patients (1,2). Other mononuclear cells such as macrophages and, to a lesser extent, B-cells are also present in active MS lesions. Together with resident CNS cells such as astrocytes and microglia, these mononuclear cells produce inflammation within the CNS and, thereby, inflict damage to both the myelin and the oligodendrocytes. Such damage may also result in irreversible axonal injury or transaction (3,4) and lead, thereby, to permanent neurological disability.

The interferons (IFNs) are a large family of secreted proteins involved in the defense of an organism against viral infections, regulation of cell growth and proliferation, and modulation of immune responses (5–8). There are two basic types of IFNs. Type I IFNs (α and β) are induced directly in response to a viral infection and are secreted principally by leukocytes (α) and fibroblasts (β). Type II IFNs (γ) are synthesized by T-lymphocytes or natural killer cells following the detection of infected cells by antigen presentation. Interferon-beta (IFNβ) is a naturally occurring glycoprotein—166 amino acids in length and with a molecular weight of 22.5 kD. It has 30% to 40% homology with the multigene IFNα family and, like the principal form of IFNα, is encoded on chromosome 9 without introns. Both IFNα and IFNβ bind to the same two-subunit receptor (IFNAR1/IFNAR2; encoded on chromosome 21), and activate a Janus kinase/signal transducer and activator of transcription (Jak/STAT) signaling pathway. This signaling pathway ultimately leads to (and with considerable complexity) the binding of interferon stimulated gene factor-3 to a short DNA sequence (approximately 10–12 bases) in the cell nucleus called the interferon stimulated response element (ISRE), which
makes up a part of several nuclear genes (6,7). Such binding leads to an activated transcription of these ISRE-containing genes, which would otherwise be expressed at low or very low levels. The ISRE also binds members of a family of interferon regulatory factors (IRFs), some of which are induced by IFNβ. The gene products induced by IFNβ include the proteins dsRNA-dependent protein kinase, 2′-5′ oligoadenylate synthase, IRF-1, IRF-2, IRF-7, the Mx family of GTPases, neopterin, major histocompatibility complex (MHC) class I molecules, and β2 microglobulin, in addition to many others (6–8). In contrast, IFNγ has no homology with IFNα or IFNβ, is located on chromosome 12 with 3 introns, binds to a different two-subunit receptor (IFNGR1/IFNGR2; encoded on chromosome 21), and activates a different (but related) Jak/STAT signaling pathway. Activation of this pathway ultimately leads to the binding of gamma activated factor to a short gamma activation sequence (GAS) encoded in the DNA of several genes and, as with the Type I IFNs, this binding enhances transcription of these GAS-containing genes such as MHC class I and II molecules, neopterin, proteosomal subunit and transfer molecules (LMP-2, LMP-7, MECL-1, TAP-1, and TAP-2), and IRF-1 (6–8).

IFNβ was the first agent demonstrated to modify unequivocally the disease course in patients with MS (9–18). There are two forms of IFNβ. The first is IFNβ-1a (Avonex and Rebif), which is genetically engineered and produced in a Chinese hamster ovary cell line. Like native human IFNβ, IFNβ-1a is a glycoprotein and has the complete 166 amino acid sequence of native human IFNβ. The pattern of glycosylation, however, will be that of the Chinese hamster. In contrast, IFNβ-1b (also genetically engineered) is produced in an Escherichia Coli cell line. Because bacteria do not glycosylate proteins, however, IFNβ-1b does not have any attached sugar molecules. In order to ensure proper folding of the IFNβ protein and to maximize its biological activity, therefore, the cysteine at position 17 has been substituted by a serine (a conservative substitution of an oxygen atom in place of a chemically similar sulfur atom). This substitution prevents the formation of some protein molecules with incorrect disulfide bonds and, thus, with low (or absent) biological activity. In addition, the N-terminal methionine (position 1) has been deleted so that the final protein is only 165 amino acids in length. Its molecular weight is only 18 kD. These chemical differences between IFNβ-1a and IFNβ-1b have certain consequences which might be clinically important, at least at a theoretical level. For example, although the two molecules seem to be equipotent in vitro, once they are combined with human serum albumin (HSA), the relative potency of IFNβ-1b decreases to approximately 10%, presumably due to a tight reversible binding with HSA. For this reason, IFNβ-1b needs to be administered in substantially larger dosages than IFNβ-1a, which might, because of the expected buffering, lead to a more stable concentration of serum IFNβ than would otherwise be possible. The higher dosage might also result in a greater propensity for the production of neutralizing antibodies (NAbs) to IFNβ or could be responsible for the ultimate disappearance of NAbs to IFNβ-1b over time. Whether any of these considerations is clinically important or not is currently unknown.

Both forms of IFNβ have been studied in clinical trials and have been shown to reduce the activity and severity of the clinical disease process (9–18). Magnetic resonance imaging (MRI) studies have also demonstrated that IFNβ reduces the number of active lesions and slows the increase in total MRI lesion volume over time (9–18). The mechanism by which IFNβ exerts these beneficial disease-modifying effects in MS is unknown but could potentially be mediated through one or more number of immunomodulatory mechanisms (19).
BIOLOGICAL CONSEQUENCES OF IFNβ ADMINISTRATION

Effects of IFNβ on T-Cell Proliferation and IFNγ Release

As discussed earlier, a key event in the pathogenesis of the MS lesion is almost certainly the activation and proliferation of auto-reactive T-cells and IFNβ is known to influence these processes. Thus, IFNβ reduces mitogen-induced proliferation of T-cells from both MS patients and healthy subjects in vitro (20). This reduction occurs regardless of either the mitogenic stimulus or the presence of IFNγ. In addition, IFNβ has been shown to reduce IFNγ release from activated T-cells in both healthy controls and MS patients (20).

Effects of IFNβ on T-Cell Migration

Another key step in the pathophysiology of MS appears to be the migration of activated T-cells across the blood–brain barrier (21). The initial step in this process is the attachment of certain proteins on the surface of the activated T-cell such as α4-integrin (also called very late antigen-4 or VLA-4) to other molecules on the endothelial surface such as vascular cellular adhesion molecule (VCAM). Because this attachment process plays such a central role in T-cell trafficking into the CNS, the effect of IFNβ on these processes may be important. For example, under the influence of proinflammatory cytokines such as IFNγ and tumour necrosis factor alpha (TNFα), vascular endothelial cells express both MHC class I and II molecules, as well as cellular adhesion molecules. These molecules help to activate and adhere leucocytes and to facilitate their migration across the vascular endothelium, and it has been shown that IFNβ downregulates IFNγ-induced class II molecule expression in human vascular endothelial cells (22).

Following such attachment, another important component of this transmigration process is the release by T-cells of matrix metalloproteinases (MMPs; also called gelatinases) in response to stimulation by the proinflammatory cytokine interleukin 2 (IL-2). MMPs cleave type IV collagen, which is part of the extracellular matrix that helps to make up the blood–brain barrier (1,2). Pretreatment of T-cells with IFNβ inhibits IL-2–dependent secretion of MMP-2 and MMP-9, and reduces MMP-dependent migration across an artificial basement membrane by up to 90%, without significantly affecting normal cell locomotion (23). Another possible mechanism for the therapeutic effect of IFNβ is its ability to downregulate IL-2 cell surface receptor expression and to reduce the affinity of IL-2 for the T-cell surface (23). Similarly, IFNβ inhibits activated leukocyte transmigration through an activated human brain microvascular endothelial cell (HB-MVEC) monolayer (24). Prestimulation of HB-MVEC, with TNFα and IFNγ, significantly promoted transepithelial migration of activated leucocytes, although, through an inhibition of TNFα, IL-1, and MMP-9 production, IFNβ is able to impede this migration (24).

IFNβ has also been reported to increase the release of soluble VCAM (sVCAM) in patients with MS (25–27). Such release might inhibit transmigration of the activated T-cells by the attachment of sVCAM to the VLA-4 antigen on the T-cell surface and might prevent, thereby, the attachment of the T-cell to the endothelial surface.

Effects of IFNβ on IL-10 Expression

It has become clear that inflammatory and other immune responses involve a complex interplay between mediators that either promote or inhibit immunological
processes, and IFNβ is known to exert inhibitory effects on several immune promoters (21). For example, IFNβ influences the expression of interleukin-10 (IL-10), a molecule released by activated T-cells, which strongly inhibits cell-mediated immune responses (28–31). Thus, incubation of peripheral blood mononuclear cells in vitro with IFNβ upregulates IL-10 mRNA expression and serum IL-10 levels are increased following injection of IFNβ into healthy subjects and MS patients (32).

**Effect of IFNβ on iNOS**

Nitric oxide is generated by an inducible nitric oxide synthase (iNOS) and has been implicated in pathogenesis of MS as contributing to the damage occurring to the myelin and to the oligodendrocytes (33). It is possible that IFNβ may reduce inflammation and cytotoxicity within the CNS of MS patients through this pathway. Thus, IFNβ has been shown to produce a selective and potent inhibition of IL-1β/IFNγ stimulated iNOS expression in cultured human astrocytes (34).

**Effect of IFNβ on NGF**

It is well known that growth factors (released by astrocytes) are important for oligodendrocyte development, maturation, and survival. In particular, nerve growth factor (NGF) stimulates adult porcine oligodendrocytes to extend processes, proliferate, and promote CNS remyelination (35). Incubation of murine astrocytes in the presence of murine IFNβ induced NGF release up to 40 times that of untreated controls (36). If similar effects were present in humans, this could be a potentially important mechanism of action for IFNβ action in MS. Interestingly, in the marmoset model of MS, NGF administration also delays the onset and reduces the severity of EAE, presumably both by downregulating IFNγ expression and by upregulating of IL-10 production in glial cells (37).

**ASSESSING THE CLINICAL AND MRI EFFECTS OF IFNβ IN MS PATIENTS**

**Evidence-Based Medicine**

The evaluation of therapeutic claims in the treatment of certain medical disorders such as MS has become increasingly complicated, requiring practicing physicians to become somewhat familiar with the fields of epidemiology and biostatistics, in order that they might understand and interpret correctly the results of individual clinical trials. Even so, however, it is difficult for physicians, engaged in busy clinical practices, to spend the time necessary for them to become truly facile with the critical analysis of clinical studies. As a consequence, considerable interest has developed in the use of so-called evidence-based medicine (EBM) to help practitioners analyze the medical literature and to promote, thereby, an improvement in the quality of the medical care received by individual patients. EBM represents a critical (and structured) evaluation of the results of clinical trials, focusing upon specific clinical questions that are (or, at least, are perceived to be) important in the management of patients. In order for EBM to be useful to practitioners, however, it is necessary for them to be familiar with the fundamentals of this analytic approach.

The EBM process is not based on consensus but, rather, involves four discrete structured steps. The first is to pose the clinical question or questions that are going
to be addressed by the assessment. These questions need to be focused and specific so that it is actually possible to provide useful answers from the medical literature. For example, a question such as “what is the role of disease modifying therapy in MS?” is too broad and cannot be answered easily from specific studies. In contrast, a focused question such as “does treatment with IFNβ reduce the relapse rate in patients with either relapsing/remitting (RR) or secondary progressive (SP) MS?” can be answered more easily from the available literature.

The second is to assemble the evidence from the medical literature, which addresses the specific questions posed at the outset. In this step, the various computerized databases need to be searched broadly and a record of the search terms used is maintained. Abstracts and papers so identified (including papers identified from the reference sections of other papers) need to be reviewed to determine their suitability for inclusion in the EBM assessment based on criteria established prior to the literature search.

The third step is to classify and interpret the evidence and the fourth is to translate this evidence into specific conclusions and recommendations. Different medical organizations use different systems for making such classifications and recommendations although, in reality, these schemes are all substantially equivalent. In the present manuscript, the system used will be, in essence, that of the American Academy of Neurology (38) and this scheme is outlined in Table 1.

Although EBM can be an extremely useful tool for practicing physicians; however, it is necessary to stress that EBM is neither designed nor intended to be the only component of the medical decision making process. In the final analysis, physicians must make individual decisions for individual patients in specific clinical circumstances. Rarely will an individual patient fit precisely into the patient population studied during the course of a clinical trial and physicians will need to decide upon a course of action based not only upon their understanding of the literature but also upon their training, clinical experience, and medical judgment. Moreover, there are many important clinical questions that cannot even be addressed by EBM because the scientifically rigorous evidence for doing an EBM assessment is often lacking. This lack of rigorous scientific evidence, however, does not imply that answers to such questions are not possible. For example, certain medical practices, such as the use of penicillin, lack high quality evidence by our current standards but, nevertheless, are undoubtedly effective. Clearly, physicians must balance their personal experience and training on the one hand and the strength of the scientific evidence on the other. In the final analysis, however, individual patients will need to be treated as individuals.

**General Methods Used to Assess IFNβ Therapy in MS**

There are four specific clinical questions that need to be addressed when considering the effectiveness of IFNβ therapy in MS. The first is whether such treatment reduces the activity of the disease process. The second is whether such treatment reduces the severity of the disease process. The third is whether there is a dose–response in the use of IFNβ as it relates to the currently available agents. And, the fourth is determining whether the presence of NAbs in the serum of patients reduces the efficacy of IFNβ and, if so, to what extent. In assembling the evidence for the efficacy of IFNβ in the treatment of MS, there are seven large (over 300 patients each), randomized, placebo-controlled trials (RCTs), three of which (9–16) studied RRMS patients and four studied SPMS patients (39–43). It might be tempting, as some authors have done (44), to consider the value of
IFNβ therapy for these two clinical forms of MS separately. However, because SPMS (by definition) always begins as RRMS, and because RRMS ultimately evolves to SPMS in most cases, it seems more reasonable to consider both of these clinical expressions of MS as part of the same underlying disease process. For example, it seems reasonable to consider the finding that IFNβ therapy reduces the attack rate in both RRMS and SPMS trial as confirmatory evidence shows that IFNβ has a beneficial effect on MS attacks. In addition, because both IFNβ-1a and IFNβ-1b, both in vitro and in vivo, seem to have very similar biological effects (45,46), it seems reasonable to consider all of the IFNβ clinical trial data in aggregate. Assembling the evidence for a possible dose–response in the use of IFNβ, there are two trials, which have studied different doses within the same clinical trial (9–15) and two head-to-head clinical trials, which have compared low-dose, once weekly, IFNβ with higher-dose IFNβ, given multiple times per week (47–49).

In order to answer each of these clinical questions, it seems reasonable to evaluate efficacy (or dose response) by clinical outcome measures (which are probably of

Table 1 Classification Scheme for Evidence and Translation of Evidence into Recommendations

<table>
<thead>
<tr>
<th>Study characteristics for classification</th>
<th>Classification of study</th>
</tr>
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<tbody>
<tr>
<td>Control group included</td>
<td>I I I ×</td>
</tr>
<tr>
<td>Representative patient population (i.e., not a highly selected sample)</td>
<td>I I × ×</td>
</tr>
<tr>
<td>Outcome assessment independent of treatment (does not need to be a blinded assessment)</td>
<td>I I × ×</td>
</tr>
<tr>
<td>Blinded outcome assessment</td>
<td>I × × × ×</td>
</tr>
<tr>
<td>Prospective trial</td>
<td>I × × × ×</td>
</tr>
<tr>
<td>Randomized triala</td>
<td>I × × × ×</td>
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<tr>
<th>Level of recommendationb</th>
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<tbody>
<tr>
<td>Translation into recommendations</td>
</tr>
<tr>
<td>Two or more Class I studies (or one convincingc Class I study)</td>
</tr>
<tr>
<td>A single Class I study</td>
</tr>
<tr>
<td>Two or more Class II studies (or one convincingc Class II study)</td>
</tr>
<tr>
<td>A single Class II study</td>
</tr>
<tr>
<td>Two or more consistent Class III studies</td>
</tr>
<tr>
<td>Data inadequate or results conflicting</td>
</tr>
</tbody>
</table>

*Yes
× No

aAlso meets standard of (i) primary outcomes clearly defined; (ii) exclusion/inclusion criteria clearly defined; (iii) dropout rate low (generally <20%) and an accounting of dropouts; and (iv) baseline characteristics between groups substantially equivalent or important covariates were prespecified in the a priori statistical analysis plan.

bA = established as effective, ineffective, or harmful; B = probably effective, ineffective, or harmful; C = possibly effective, ineffective, or harmful; U = data inadequate or conflicting.

cLower limit of the 95% confidence interval for the odds ratio ≥2.0, if a single study.
immediate importance to patients), in conjunction with MRI measures (which are unequivocally blinded and, therefore, less subject to bias). Thus, disease activity can be evaluated by both clinical activity measure (e.g., the attack rate, time to first attack, or attack-free status), and an MRI activity measure (e.g., gadolinium-enhancing lesions, new T2 lesions, or a combination of these two measures). Similarly, disease severity can be evaluated by a clinical severity measure (e.g., confirmed one-point progression on the EDSS scale, the integrated EDSS scale, or the MS functional composite) and by an MRI severity measure (e.g., the total T2 volume of disease, the number or volume of black holes, or atrophy). For the purposes of this assessment, studies have been classified using a four-tiered system and recommendations derived in accordance with the AAN process, as outlined in Table 1 (38). In addition, because the minimum Type I (\(\alpha\)) error rate for an experimental observation with \(P = 0.05\) can be calculated, and is actually 13\% (38,50), observations with \(0.01 \leq P \leq 0.05\) have been regarded as only marginally significant.

### Assessment of the Efficacy of IFNβ

The classification of the different clinical trials of IFNβ with respect to the questions about efficacy is shown in Table 2. All of these trials were placebo-controlled RCTs and, therefore, all provided class I data with respect to both activity and severity measures of efficacy. The results of the different clinical trials for IFNβ, are shown in Tables 3 and 4. All of these trials found a beneficial effect of IFNβ on clinical attack rates and in six of the seven this effect was statistically convincing (Table 3). Similarly for MRI activity rates, all of these seven trials showed a beneficial effect and, again, in six of the seven this effect was statistically convincing (Table 3). In sum, therefore, there is a very consistent and convincing evidence for a benefit of IFNβ on MS disease activity, regardless of whether this is measured clinically or by MRI. In contrast, the benefit of IFNβ on the clinical severity outcome measure of confirmed EDSS progression is less convincing. Thus, only three of the seven trials found a significant benefit and, in only one (39), this effect was statistically convincing. Unfortunately, even in the case of this single convincing trial, the finding is equivocal. Thus, when an attempt was made to replicate this European experience (39) in North America (40), the beneficial effect of IFNβ on confirmed EDSS progression was not confirmed (Table 3). Among other things, such an observation clearly demonstrates the importance of requiring either more than one class I study

<table>
<thead>
<tr>
<th>IFNβ trial</th>
<th>Size</th>
<th>Controls</th>
<th>Randomized</th>
<th>Prospective</th>
<th>Blinded</th>
<th>Class</th>
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<td>372</td>
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<td>•</td>
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<td>I</td>
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<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>I</td>
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<tr>
<td>E Betaseron, SPMS (39)</td>
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<td>I</td>
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<tr>
<td>NA Betaseron, SPMS (40)</td>
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<td>•</td>
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<td>I</td>
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<td>506</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>I</td>
</tr>
</tbody>
</table>

*Yes

**Abbreviations:** IFNβ, interferon beta; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.
or overwhelming evidence from a single study to support a Level A conclusion for established efficacy (Table 1). However, despite the fact that the data for IFNβ is less convincing on this outcome, there are several reasons to believe that IFNβ also reduces the disease severity in patients with MS, at least in those patients who are still continuing to experience attacks. First, even in the original IFNβ-1b trial, there was a nonsignificant trend in favor of therapy of similar magnitude (and not significantly different) from that found in the other RRMS trials (9–16). Second, in the nonsignificant SPMS trials of Betaseron and Rebif (39,40) there seemed to be a benefit to therapy on disease severity outcomes in those patients who were still experiencing relapses. This observation suggests that patients in the inflammatory phase of their illness are still deriving benefit from IFNβ therapy. Third, and most importantly, when MRI was used to the effect of IFNβ on disease severity, six of the seven trials demonstrated a statistically convincing benefit from IFNβ (Table 3).

On the basis of this evidence, therefore, IFNβ seems well established as an effective treatment for reducing MS disease activity (Level A conclusion) and as a probably effective treatment for reducing MS disease severity (Level B conclusion).

**Assessment of the Dose–Response of IFNβ Effects**

Four separate lines of evidence to suggest that the total weekly dose of IFNβ, the frequency of IFNβ administration, or both are important factors in the treatment of MS with IFNβ. First, a large body of experimental data suggests that higher doses of IFNβ produce greater biologic effects both in vitro and in vivo (51).

Second, comparing the results of the different clinical trials of IFNβ in MS generally supports the view that higher doses or more frequently administered, IFNβ has greater efficacy than lower doses administered less often (Table 4). When comparing the various clinical and MRI outcomes of these different therapeutic trials, however, it is important to recognize that the dosages of IFNβ [expressed in millions of international units (MIUs) of IFNβ activity] are reported in different units in the different publications. Thus, each pharmaceutical company used a different assay to measure IFNβ activity and, as a result, the MIU scales are not directly comparable between the different agents. However, a rough comparison can be made by noting that Avonex

---

**Table 3** Results (Improvement) in Different Outcomes of the Different Clinical Trials of Interferon Beta

<table>
<thead>
<tr>
<th>IFNβ trial</th>
<th>Clinical activity</th>
<th>MRI activity</th>
<th>Clinical severity</th>
<th>MRI severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betaseron, RRMS (9–11)</td>
<td>●</td>
<td>●</td>
<td>ns</td>
<td>●</td>
</tr>
<tr>
<td>Avonex, RRMS (12,13)</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>ns</td>
</tr>
<tr>
<td>Rebif, RRMS (14–16)</td>
<td>●</td>
<td>●</td>
<td>○</td>
<td>●</td>
</tr>
<tr>
<td>E Betaseron, SPMS (39)</td>
<td>●</td>
<td>●</td>
<td>ns</td>
<td>●</td>
</tr>
<tr>
<td>NA Betaseron, SPMS (40)</td>
<td>●</td>
<td>●</td>
<td>ns</td>
<td>●</td>
</tr>
<tr>
<td>Avonex, SPMS (43)</td>
<td>●</td>
<td>●</td>
<td>ns</td>
<td>●</td>
</tr>
<tr>
<td>Rebif, SPMS (41,42)</td>
<td>●</td>
<td>●</td>
<td>ns</td>
<td>●</td>
</tr>
</tbody>
</table>

● Significant ($P < 0.01$)  ○ Marginally significant ($P = 0.01–0.05$)

*Abbreviations: IFNβ, interferon-beta; MRI, magnetic resonance imaging; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; ns, not significant.*
### Table 4: Principal Outcomes During Years 1 and 2 (Compared to Placebo) in the Randomized, Placebo-Controlled Trials of Interferon Beta in Relapsing–Remitting Multiple Sclerosis

**Dosing and other information**

<table>
<thead>
<tr>
<th>Total weekly dose of IFNβ (in μg)</th>
<th>175</th>
<th>22</th>
<th>30</th>
<th>44</th>
<th>66</th>
<th>875</th>
<th>132</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weekly dose of IFNβ (in MIU)</td>
<td>5.6</td>
<td>6</td>
<td>6</td>
<td>12</td>
<td>18</td>
<td>28</td>
<td>36</td>
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</tbody>
</table>

**Dose schedule (route)**

<table>
<thead>
<tr>
<th>Mean EDSS at entry</th>
<th>2.9</th>
<th>2.7</th>
<th>2.4</th>
<th>2.6</th>
<th>2.5</th>
<th>3.0</th>
<th>2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDSS range at entry</td>
<td>0–5.5</td>
<td>0–5.0</td>
<td>1–3.5</td>
<td>0–5.0</td>
<td>0–5.0</td>
<td>0–5.0</td>
<td>0–5.0</td>
</tr>
</tbody>
</table>

**Outcome measures of disease activity**

<table>
<thead>
<tr>
<th>Relapse rate, 1 yr</th>
<th>−15%</th>
<th>0%</th>
<th>−10%</th>
<th>−19%</th>
<th>−33%</th>
<th>−33%</th>
<th>−37%</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Relapse-free patients, 1 yr</td>
<td>−</td>
<td>0%</td>
<td>−</td>
<td>+11%</td>
<td>+59%</td>
<td>−</td>
<td>+87%</td>
</tr>
<tr>
<td>Median time to first relapse</td>
<td>+18%</td>
<td>−</td>
<td>+31%</td>
<td>−</td>
<td>+70%</td>
<td>+86%</td>
<td>+113%</td>
</tr>
<tr>
<td>Relapse rate, 2 yr (ITT)</td>
<td>−13%</td>
<td>−</td>
<td>−18%</td>
<td>−</td>
<td>−29%</td>
<td>−34%</td>
<td>−32%</td>
</tr>
<tr>
<td>% Relapse-free patients, 2 yr</td>
<td>+29%</td>
<td>−</td>
<td>+42%</td>
<td>−</td>
<td>+69%</td>
<td>+95%</td>
<td>+100%</td>
</tr>
<tr>
<td>Median MRI attack rate, 2 yr</td>
<td>−67%</td>
<td>−</td>
<td>−33%</td>
<td>−</td>
<td>−67%</td>
<td>−83%</td>
<td>−78%</td>
</tr>
</tbody>
</table>

**Outcome measures of disease severity**

| Confirmed progression (1 EDSS point), 2 yr | −     | − | −37% | − | −19% | −29% | −30% |
| Median % change in MRI BOD, 2 yr         | −5.1% | − | −6.7% | − | −12.1% | −17.3% | −14.7% |
| Final EDSS ≥ 1 point more than baseline  | 0%   | − | −32% | − | −     | −31% | −     |

---

*a* Percentage reductions (or increases) have been calculated by dividing the reported rates in the treated group by the comparable rates in the placebo group, except for MRI disease burden which was calculated as the difference in % change between the treated and placebo groups.

*b* Dosages of IFNβ as expressed in MIUs of IFNβ activity, as reported in the published papers. Each company, however, used a slightly different assay to measure IFNβ activity and, therefore, the MIU scales are not directly comparable between the different publications.

*c* MRI attack rate (activity) was measured differently in different trials. The Betaseron (9–11) and Rebif (14,15) trials include the number of new, recurrent, and enlarging T2 lesions but not Gadolinium (Gd) enhancement; the Avonex (12,13) trial used only the number of Gd enhancing lesions.

*d* Betaseron trial (9–11).

*e* Rebif trial (17).

*f* Avonex trial (12,13).

*g* Rebif trial (14,15).

*h* Summary basis for FDA approval (51).

\[ P \leq 0.05 \]

\[ P \leq 0.01 \]

\[ P \leq 0.001 \]

\[ P \leq 0.0001 \] compared with controls.

**Abbreviations:** IFNβ, interferon-beta; qod, every other day; s.c., subcutaneous; qw, once per week; i.m., intramuscular; tiw, three times per week; EDSS, Kurtzke expanded disability status scale; ITT, intention to treat analysis; MRI, magnetic resonance imaging; MS, multiple sclerosis; BOD, burden of disease.
and Rebif (identical molecules) are equivalent on a microgram for microgram basis, and by further noting that six MIU of Avonex is approximately equivalent to seven to nine MIU on the Betaseron scale (38). Using these conversions, the entries (columns) in Table 4 have been arranged in approximate ascending order of weekly IFNβ dose (from left to right) and, from perusal of this Table, it can be appreciated that for most outcomes, the higher doses of IFNβ are associated with greater therapeutic effects (Table 4).

Third, in both clinical trials that compared, within the same trial, two different IFNβ doses (9–11,14–16), the higher dose was consistently better than the lower dose for most clinical and MRI outcomes, even though only some of these between group comparisons were statistically significant. Nevertheless, the consistency of the apparent dose-effect is notable.

Fourth, and perhaps most importantly, in the head-to-head trials, which compared high-dose (more frequent) IFNβ preparations directly to low-dose (once weekly) IFNβ, the high dose (more frequent) arm was significantly favored with respect to clinical and MRI measures of efficacy (47–49). As is shown in Table 5, this was the case both for the INCOMIN trial (47), which compared standard-dose Betaseron (250 μg, s.c., qod) to standard dose Avonex (30 μg, i.m., qw) and for the EVIDENCE trial (48,49), which compared high-dose Rebif (44 μg, s.c., tiw) to standard dose Avonex (30 μg, i.m., qw). Because outcome assessment in the INCOMIN trial was blinded for MRI and not clinical assessments, this trial provides a mixture of class I data for MRI outcomes and class III for clinical outcomes (Table 4). In contrast, because the EVIDENCE trial used blinded outcome assessment for both clinical

| Table 5 Evidence Classification of the Head-to-Head Trials Assessing the Role of Interferon Beta Dose in the Treatment of Multiple Sclerosis |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|
| Classification             | Size | Controls | Randomized | Prospective | Blinded | Class |
| INCOMIN, RRMS (47)         | 188  | •        | •           | •            | •/×      | I/III |
| EVIDENCE, RRMS (48,49)     | 677  | •        | •           | •            | •       | I     |

Results:
- INCOMIN compared Betaseron and Avonex; EVIDENCE compared Rebif and Avonex.
- • Yes
- •/× Blinded for some outcomes but not others
- $ Significant ($P < 0.01$)
- $ Marginally significant ($P = 0.01–0.05$)
- Blank cells = not reported
- Statistical significance indicates a better outcome for the Betaseron or Rebif arms compared to the Avonex arm in each trial.

Abbreviations: MRI, magnetic resonance imaging; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; ns, not significant.
and MRI measures, this trial provides class I data for each (Table 4). The only non-significant difference between high- and low-dose was in the EVIDENCE trial for the clinical severity outcome of confirmed EDSS progression, which may have been due, in part, to the short duration of follow-up in this trial (Table 4).

On the basis of this evidence, therefore, it seems that there probably is a dose–response to the clinical use of IFNβ in the treatment for relapsing MS (Level B conclusion). However, because all of the currently available head-to-head data confounds the effects of total weekly dose with that of the frequency of administration, it is possible that some (perhaps, all) of this dose–response may be due to the frequency of IFNβ administration rather than total dose.

Assessment of the Effect of Neutralizing Antibodies to IFNβ

Most IFNβ-treated patients will develop antibodies to the IFNβ molecule (52). Two different kinds of antibodies are produced. The first (and most prevalent) type of antibodies are called “binding” (BAb) because they do not necessarily interfere either with binding of IFNβ to its receptor or with its receptor-mediated functions. The second type of antibodies are called “neutralizing” (NAbs) because they interfere with receptor binding and/or receptor-mediated functions. The NAbs are, thus, a subset of the BAbS. The difference between these antibodies might be explained if, as is often suggested, NAbs are attached to the receptor-binding region of the IFNβ molecule and BAbS are attached to other, less critical, regions. However, the fact that some BAbS seem not to interfere with the receptor binding might be viewed as surprising, given the huge difference in size between the two molecules. For example, IgG has a molecular weight between 150 to 170 kD (compared to the 18–23 kD for IFNβ), and it is hard to imagine completely normal receptor interactions with such a large moiety appended anywhere to the IFNβ molecule. Nevertheless, it is clear that some BAbS seem to have little impact on interferon activity, at least as we currently measure it. It is also possible that BAbS might increase the clearance of IFNβ through the reticuloendothelial system and thereby, lower serum IFNβ levels (a circumstance which would not be detected by current NAb assays). However, the principal antibodies likely to impact IFNβ activity are the NAbs, which potentially could diminish the biological activity of administered IFNβ and reduce, thereby, the effectiveness of therapy. As an example of such an effect, the presence of NAbs has been associated with a complete loss of detectable IFNβ activity in the serum (53).

Several different techniques can be used to detect the presence of antibodies to IFNβ in the serum of patients (54). Enzyme-linked immunosorbent assays (ELISAs) measure antibodies to any of the IFNβ epitopes, and will measure both BAbS and NAbs. The MxA assay measures a serum protein that is induced by IFNβ and which is reduced, in the presence of NAbs, to IFNβ. Cytopathic effect (CPE) assays detect NAbs by demonstrating the neutralization of IFNβ-induced inhibition of viral-mediated cell lysis. Currently, most diagnostic laboratories utilize the CPE assay although both are problematic and are sensitive to the specific conditions in which the assays are performed. Perhaps, both assays should be undertaken together. For example, in a recent reanalysis (55) of the pivotal IFNβ trial (9–11), all the patients who were eventually NAb-positive by both assays were in one of the two treatment groups. In contrast, either assay, by itself, had a 2% to 4% false positive rate as judged by the other assay (55). Also, it is probably preferable to measure NAbs using a two step method where patient sera are first analyzed by ELISA for the presence of BAbS, and then positive sera are screened for NAbs using a CPE (or MxA) assay (56).
As one example of title potential effect of NAbs on efficacy, in the phase III Betaseron trial (9–11), 38% of patients in the high dose arm became NAb positive (defined as two consecutive positive titers three months apart) after two years. When NAb-positive and NAb-negative patients were analyzed separately, the NAb-positive patients seemed to behave more like the placebo-treated patients with respect to their attack rate (52). Interpretation of this observation, however, is not as straightforward as it might seem. First, many of the patients analyzed in this fashion did not become NAb-positive until late in the trial and a large percentage of patients who became NAb-positive ultimately became NAb-negative, at least temporarily (54). Thus, in the high-dose arm of this trial, 51% of the CPE-positive patients and 65% of the MxA-positive patients reverted to NAb-negative status at some time. Second, it is not clear that clinical attacks during a patient’s NAb-negative period should be attributed to the NAb-positive rate. Third, the relevance of the biological activities neutralized by NAbs to the effect that IFNβ has on MS is uncertain. Fourth, antigen–antibody complexes are also well known to modulate immune functions. These immune effects will be independent of the receptor-mediated functions of IFNβ and will presumably be more conspicuous in patients with high antibody titers. And finally, the long-term consequences of NAbs are unknown. For example, in the long-term follow-up study of Canadian patients who took part in the high-dose arm of the original IFNβ-1b trial, almost 80% of patients who were NAb-positive during the study had become NAb-negative after eight years (57). These considerations are relevant (more or less) to all of the NAb data that is currently available and this will necessarily make any analysis of the clinical impact of NAbs quite complicated.

Nevertheless, despite such complexities, it is still difficult to imagine that persistently high NAb titers to IFNβ would not have some deleterious effect on the clinical efficacy of IFNβ. And, indeed, when all of the evidence is assembled, there does seem to be an impact of the presence of NAbs on outcome, especially for MRI outcomes (Table 6). Although the effects on clinical measures (especially clinical severity) are less convincing, every study reported that the MRI outcomes for activity and severity were better (although not always significantly so) in the NAb-negative group (Table 6). It, therefore, seems only reasonable to conclude that the presence of NAbs is probably deleterious. However, even if it is conceded both that NAbs are more prevalent with high-dose (more frequent), subcutaneous IFNβ and that NAbs adversely impact the effectiveness of therapy, it is still unclear how this information would (or should) affect therapeutic decisions. For example, it is not clear whether the presence of NAbs would completely abrogate the clinical effects of IFNβ (as opposed to merely attenuating it). Also, importantly, it is not clear whether such a deleterious effect of NAbs would offset the improved efficacy reported with high-dose (more frequent) IFNβ. This is a fundamentally different question than asking whether NAbs have a clinical impact or not and, unfortunately, there is a paucity of actual data available to answer this question. The only two trials, which have comparative data of this type, are the EVIDENCE (48,49) and the INCOMIN (47) trials, and it is possible that these trials, particularly the EVIDENCE trial, are too short to provide adequate answers to the question—a point that has also been made by other investigators. In both these trials, however, even the NAb-positive patients in the high-dose (more frequent) IFNβ arms had fewer relapses than the arm receiving low-dose (once weekly) IFNβ (47–49). Thus, at least over the first two-years of treatment, all of the available data suggests that title balance favors the effective therapy, even if this therapy is associated with a
greater propensity to produce NAbs. Whether this relative advantage of high-dose (more frequent) therapy is sustained in the long term is a theoretical discussion (i.e., not evidence based), as is a discussion, which will clearly need to include a consideration of the propensity of NAbs to disappear spontaneously over time (55,57).

In the phase III Avonex trial, only 22% of patients developed NAbs (defined as “once positive always positive”) after two years of therapy (12). However, in a separate study on a newly formulated IFN\(_b\)-la product, only 6% of the IFN\(_b\)-la treated patients developed NAbs (56). The basis for this marked difference in immunogenicity between formulations has never been adequately explained or rationalized. A portion of the difference in NAb-positivity between Betaseron and Avonex trials could conceivably relate to the difference in total dose of IFN\(_b\) administered to patients.

In the phase III Rebif trial (14–16), although both the low-dose and high-dose groups developed NAbs to IFN\(_b\), the observed rates of NAb-positivity were considerably greater in the low-dose group compared with the high-dose arm (24% and 13%, respectively). This same observation was also made in the trial of Rebif in SPMS (41,42). Although such observations are difficult to rationalize clearly, they suggest that dosage is not the explanation for the reported differences in immunogenicity between products. However, in the phase III Rebif trial (14–16), although both the low-dose and high-dose groups developed NAbs to IFN\(_b\), the observed rates of NAb-positivity were considerably greater in the low-dose group compared with the high-dose arm (24% and 13%, respectively). This same observation was also made in the trial of Rebif in SPMS (41,42). Although such observations are difficult to rationalize clearly, they suggest that dosage is not the explanation for the reported differences in immunogenicity between products. However, they do suggest that intramuscular administration (or weekly dosing) is less immunogenic than subcutaneous administration (or frequent dosing) and that IFN\(_b\)-la is less immunogenic than IFN\(_b\)-1b.

The apparently lower immunogenicity of IFN\(_b\)-1a compared with IFN\(_b\)-1b may relate to a number of factors. First, because IFN\(_b\)-1a is glycosylated, it may be less immunogenic compared with the nonglycosylated IFN\(_b\)-1b (63–65). In addition, it is possible that the nonglycosylated IFN\(_b\)-1b forms aggregates, which have less (or no) biological activity (63–65). It is also possible that any such aggregate forms might potentially lead to an increased immunogenicity. However, whether IFN\(_b\)-1b actually forms aggregates is unclear. As discussed earlier, the reduced

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Clinical activity</th>
<th>MRI activity</th>
<th>Clinical severity</th>
<th>MRI severity</th>
<th>Class</th>
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<td>IFN(_b)-1b MS Study Group (9–11,58)</td>
<td>1996</td>
<td>+(^b)</td>
<td>+ (ns)</td>
<td>− (ns)</td>
<td>+ (ns)</td>
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<td>MSCRG (12,13,59)</td>
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<td>− (ns)</td>
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<td>PRISMS (14–16)</td>
<td>2001</td>
<td>+(^c)</td>
<td>+(^d)</td>
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<td>+ (ns)</td>
<td>+(^d)</td>
<td>− (ns)</td>
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<td>II</td>
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<tr>
<td>INCOMIN (47)</td>
<td>2002</td>
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<td>+ (ns)</td>
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<td>II</td>
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<td>EVIDENCE (48,49)</td>
<td>2002</td>
<td>+ (ns)</td>
<td></td>
<td>+(^d)</td>
<td></td>
<td>II</td>
</tr>
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<td></td>
<td></td>
<td>+(^c)</td>
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<tr>
<td>Frank et al. (62)</td>
<td>2004</td>
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<td>+(^c)</td>
<td></td>
<td></td>
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</tbody>
</table>

Abbreviations: MRI, magnetic resonance imaging; IFN\(_b\), interferon beta; ns, not significant.

Table 6 The Effect of Neutralizing Antibodies on Clinical and Magnetic Resonance Imaging Outcomes in Multiple Sclerosis Therapeutic Trials

\(^a\) + outcome worse in NAb-positive group than NAb-negative group; − outcome worse in NAb-negative group than NAb-positive group.

\(^b\) \(P < 0.05\).

\(^c\) \(P < 0.01\).

\(^d\) \(P < 0.001\).
potency of the IFNβ-1b molecule compared with IFNβ-1a seems to be due to a reversible binding to albumen in the serum rather than to the formation of aggregates. Another factor that might produce a higher rate of NAb formation, as discussed earlier, is a subcutaneous route of administration of IFNβ. Thus, in contrast to muscle, the skin is quite active immunologically, with resident antigen presenting cells to mediate both humeral and cellular immune responses. Such a circumstance might potentially predispose to the formation of NAb. Certainly, the results of the EVIDENCE trial (48,49), where the difference in NAb prevalence between the Avonex and Rebif arms was quite striking (2% and 25%, respectively), strongly suggests that either route or frequency of IFNβ-la administration makes a difference.

In conclusion, on the basis of several class I studies, treatment of MS patients with IFNβ (Avonex, Betaseron, or Rebif) is associated with the production of NAb to the IFNβ molecule (Level A conclusion). It seems likely, however, that the rate of NAb production is less with IFNβ-la treatment than with IFNβ-1b treatment although, because of the variability of the data, the magnitude of the actual difference is difficult to determine (Level B conclusion). The biological effect of NAb is uncertain, although it seems probable that their presence is associated with a reduction in clinical effectiveness of IFNβ treatment (Level B conclusion). It is also probable that there is a difference in immunogenicity between subcutaneous and intramuscular routes of administration (Level B conclusion), although it is possible that other factors (e.g., frequency of administration, acidity, etc.) may account for the observed differences. However, the greater prevalence of NAb with high-dose (more frequent) IFNβ, together with the potentially reduced effectiveness of INFβ in the presence of NAb, probably does not offset the improved efficacy of the high-dose (more frequent) formulations, at least during the first two years of therapy.

CONCLUSIONS

In conclusion, the introduction of INFβ therapy into the clinical management of MS patients in the early 1990s represented a great stride forward in the treatment of MS. This was the first therapeutic agent shown to have unequivocally beneficial effects on the biological activity of the human illness. No similar agent had ever been available to practitioners ever since the illness was initially described in the mid-18th century by Charcot. Indeed, now a decade after its introduction, the therapeutic efficacy of IFNβ now seems particularly well established on the basis of seven large, independent, multi-center trials of this agent. These trials have consistently demonstrated IFNβ to have beneficial effects on both the activity and severity of the underlying illness. It is important to recognize, however, that, although IFNβ represents an important first step in the treatment of MS patients, it is only a partially effective therapy. In order to actually cure the illness or even to substantially improve patient outcome, we will need considerably better agents than we have at the current time. Perhaps, the recent introduction of natalizumab (a humanized monoclonal antibody directed against the VLA-4 antigen on the T-cell) may represent a development and the final results of the two pivotal trials of this agent are awaited with eager anticipation. Perhaps, also, increasing the dose of IFNβ or using it in combination with other agents such as natalizumab may yield even better patient outcome than are possible with the single agent therapies currently available. All of these possibilities are being actively investigated at the present time. Finally, and hopefully, improvements in our understanding of the fundamental physiological and biological roles of
IFNβ, both in health and in the pathogenesis of MS, will lead to the development of considerably improved treatments for MS in the future.

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Yang Mao-Draayer and Hillel S. Panitch
Department of Neurology, University of Vermont College of Medicine, Burlington, Vermont, U.S.A.

INTRODUCTION

The past 15 years have seen a revolution in our understanding and management of many neurologic diseases, including multiple sclerosis (MS). The US Food and Drug Administration (FDA) approved the first of the interferon (IFN) preparations, IFNβ-1b (Betaseron®) for MS in 1993, followed by glatiramer acetate (GA, Copaxone®) in 1995, intramuscular IFNβ-1a (Avonex®) in 1996, then mitoxatrone (Novantrone®) and subcutaneous IFNβ-1a (Rebif®) in 2000 and 2002, respectively. Treatment with GA or the interferons has become the standard of care for patients with relapsing–remitting multiple sclerosis (RRMS). FDA approval for GA was granted on the basis of two pivotal double-blind, placebo-controlled clinical trials (1,2) that convincingly demonstrated the ability of GA to reduce relapse rates in MS and, to a lesser extent, slow the progression of neurologic disability. In addition, the clinical success of GA and the interferons has generated a wealth of basic research and clinical trials that have helped to clarify their mechanisms of action and extend their clinical applications. The purpose of this chapter will be to review the pivotal trials that led to the approval of GA, subsequent studies that established its effect on magnetic resonance imaging (MRI) activity, unsuccessful trials defining the limitations of GA therapy, ongoing clinical trials involving using GA in new ways, insights into the immunological mechanism of action of the drug, and current thinking about the appropriate place of GA in the MS therapeutic armamentarium.

CLINICAL STUDIES OF GA

Clinical Trials of GA in RRMS

Phase II Pilot Study

Following an open-label dose finding study in 16 patients, in which GA was shown to be safe, and the standard dose of subcutaneous 20 mg/day is established (3), Bornstein et al. (1) undertook a randomized, double-blind, placebo-controlled phase II trial in 50 patients with relapsing–remitting MS. Patients were enrolled as
matched pairs, stratified by age, sex, relapse rate, and Kurtzke disability status score (DSS), and were followed at regular intervals by blinded examiners. The results were remarkable with 62 confirmed attacks in the placebo group compared to only 16 in the GA-treated group over two years, corresponding to a reduction in annualized relapse rate from 1.35 to 0.30, a highly significant result. Patients with the lowest disability scores (DSS 0–2) responded best, with 27 attacks in the placebo-treated patients, but only four among those treated with GA, suggesting that treatment should be initiated as early as possible. A beneficial effect was also found for progression of disease, with significant differences between the active drug and placebo groups in proportion of progression-free patients and time to confirmed progression. This trial also established the tolerability of GA, which has been confirmed in all later trials.

Phase III Pivotal Trial and Open-Label Extension

Before a definitive clinical trial could be undertaken, it was necessary to standardize the preparation of the drug and to produce it in large enough quantities to perform a phase III multicenter study. GA used in previous studies had been prepared in small batches that, despite their constant amino acid composition, varied widely in molecular weight and ability to suppress experimental allergic encephalomyelitis (EAE). By 1991, Teva Pharmaceutical Industries Ltd. (Petah Tiqva, Israel) had succeeded in standardizing the manufacturing process to produce large batches of consistently active drug, acceptable to the FDA for use in a pivotal trial. The formulated product, Copaxone, contained 20 mg/mL of GA with an average molecular weight of 4700 to 13,000 daltons, and 40 mg/mL of mannitol to increase stability and solubility. Each batch of the commercial product was tested for uniformity, and was capable of suppressing EAE.

The phase III pivotal trial, conducted in the United States from 1991 to 1994 (2,4), included 251 patients, 18 to 45 years of age, with clinically definite relapsing–remitting MS, expanded disability status scores (EDSS) from 0 to 5, and a history of two or more relapses in the two years prior to entry. They were randomized to receive either 20 mg of GA, or a placebo, by daily subcutaneous injection for 24 months. The primary outcome measure was a comparison of relapse rates in the two groups, with relapses being carefully defined as appearance of a neurologic abnormality, persisting at least 48 hours, following a period of stability or improvement lasting at least 30 days. Only relapses confirmed by objective changes on neurological examination were counted in the analysis. Secondary outcome measures included proportion of relapse-free patients, time to first relapse, sustained progression (defined as an increase of one or more points on the EDSS persisting for at least three months), and mean EDSS change in the two groups. Patients were instructed in self-injection of the drug, and were examined at three-month intervals by a blinded examiner and a blinded treating neurologist who was responsible for steroid treatment, if necessary, for confirmed relapses.

The placebo and active drug groups were well matched except for mean EDSS, which was slightly higher in the GA treated patients. Approximately equal numbers of patients withdrew from both groups over two years, which did not appreciably affect the intention-to-treat analysis. The principal outcome of the study was a 29% reduction ($P = 0.007$) in the mean two-year relapse rate from 1.68 in the placebo group to 1.19 in the GA group (2). Patients in both groups with higher EDSS scores at entry had more relapses during the trial, but the therapeutic effect was greatest in
patients with EDSS scores of 0 to 2, who showed a 33% reduction in relapse rate, while those with higher scores had only a 22% reduction. The effect of GA on progression was significant as measured by the proportion of patients who improved or worsened by one or more EDSS points compared to patients treated with placebo. Patients on GA tended to improve or remain stable, whereas those on placebo tended to worsen. Mean change in EDSS from baseline was also statistically better in the GA treated group, although the difference was numerically small, and of dubious clinical significance. There was no significant effect on the proportion of progression-free patients defined by EDSS sustained for at least three months.

After FDA approval of GA in 1995, the phase III trial was extended in double-blind, placebo-controlled fashion up to 35 months with over 80% of patients (99 on GA and 104 on placebo) remaining in the study. This extension permitted the collection of additional data that confirmed and strengthened the results of the core study (4). Relapse rates, recalculated for the entire trial period, showed a 32% reduction in favor of GA \((P = 0.002)\). The proportion of relapse-free patients and median time to first relapse also became statistically significant. All patients who had been relapse-free in the original study remained so in the extension. When proportions of patients who improved on GA and worsened on placebo by one or more EDSS points were tested, the significant differences favoring GA were maintained, and Kaplan-Meier curves generated for patients who progressed by 1.5 EDSS points showed a significant treatment effect on slowing of disability progression. In the placebo group, 41.6% of patients worsened by 1.5 points or more, whereas in the GA group only 21.6% worsened \((P = 0.001)\). The result was not complicated by intercurrent relapses, since EDSS scores obtained during relapses and for 30 days afterward were excluded from the analysis. Significant results were also obtained for progression by \(\geq 1.0\) EDSS point (placebo 59.2% vs. GA 42.4%, \(P = 0.008\)), but the data for a 1.5 point change were considered more robust and less subject to examiner variability (5). When a somewhat different data analysis, the integrated disability status score, or area under the curve (6) was applied, this combined measure of relapse and progression over time also showed a positive effect of the drug (7). Thus, the therapeutic effects of GA were maintained for up to 35 months, and most of the outcome measures suggested that its clinical efficacy persisted and improved with time.

Of the original 251 patients, 208 chose to continue on active drug for an indefinite period of time, and to continue regular follow up. A six-year extension study was published in 2000 (8). The subjects were approximately evenly divided between those originally assigned to GA or placebo. Patients were examined every six months, using the same outcome measures as in the blinded portion of the study, in most cases by the same examiners. Admittedly, there are problems in interpreting such a study without a placebo control group, and attempts have been made to use natural history controls with questionable success. Nevertheless, some observations could be made: the majority of patients (152/208 or 73%) remained on treatment, had low rates of relapse and progression, and continued to tolerate the drug well. The annualized relapse rate for patients continuously on active drug (median 5.83 years) was 0.42 attacks per year, and for the 6th year the relapse rate was 0.23. Similar rates of decline were seen in the group originally randomized to placebo, although their annualized relapse rate was higher. Meanwhile, the majority showed no evidence of EDSS progression, indicating that cessation of relapses did not signal conversion to secondary progressive MS. At eight years, 142 (56.6%) of the original patients remained in the study (9). The annual relapse rate declined to
approximately 0.2 and there was a significant difference in EDSS scores between patients originally randomized to GA compared with those who switched to GA after an average of 30 months on placebo (65.3% vs. 50.4% stable or improved respectively, \(P = 0.0263\)), supporting early initiation of GA treatment.

At 10 years, another analysis was performed in the 232 patients who had been treated with GA at any time, and an attempt was made to contact and examine patients who had discontinued the study (10). The intention-to-treat cohort continued to experience a remarkably low annual relapse rate of 0.2 to 0.25, or one relapse every four to five years, without a concomitant increase in EDSS scores. Patients treated continuously with GA (\(n = 108\)) were compared with those who discontinued the study, but returned for long-term followup (\(n = 50\)). As expected, outcomes were better in those on continuous therapy. EDSS values at GA initiation, 4 years, and 10 years were 2.56, 2.55, and 3.06 with continuous GA treatment, compared with 2.84, 3.77, and 5.11 in patients who withdrew with long-term followup (\(P < 0.0001\) for EDSS at year 10). Similarly, the proportions of patients who reached EDSS milestones of 4, 6, and 8 were greater in the group that withdrew from the study. This was the longest prospective, organized evaluation of continuous immunomodulatory therapy ever carried out in MS. Although these observations were uncontrolled, and selection bias inevitably played a role in the outcome, it is clear that early and extended treatment with GA offers nearly 50% of relapsing–remitting MS patients an excellent outcome over a decade or more. The challenge now, as it is for all MS disease modifying agents, is to predict which patients are most likely to respond.

**Phase III Oral Trial (CORAL)**

A large multinational trial of oral GA, given the acronym “CORAL”, has also been conducted, based on observations in experimental animals that oral GA could suppress EAE, and suppression could be adoptively transferred by antigen-specific T-cells obtained from treated animals (11). Oral GA has been shown to induce suppressive Th2 cytokines including IL-10 and TGF-\(\beta\) (but not IL-4), and to inhibit secretion of IFN-\(\gamma\), similar to its mechanism of action when given parenterally. Although induction of oral tolerance with whole myelin did not succeed in suppressing disease activity in MS (12,13), GA was thought perhaps to have greater potential because it is not encephalitogenic in animals, and its efficacy in MS by the parenteral route had already been established (14). Thus, it appeared to be a very promising treatment modality, much more acceptable to patients than the daily injections required with parenteral GA. In addition, the availability of an effective oral therapy for MS would greatly facilitate trials in combination with other drugs.

CORAL was a double blind, placebo controlled, randomized multinational study including 1644 patients with clinically definite relapsing–remitting MS, one or more attacks in the year prior to entry, and EDSS scores of 0 to 5. They were randomized to either 5 or 50 mg of GA, or matched placebo tablets, taken daily for 56 weeks, and were evaluated clinically every two months. MRI scans were performed at baseline and at study completion, and a subset of over 400 patients had bimonthly gadolinium-enhanced scans. The primary outcome measure was the number of documented relapses per patient per group. Numerous clinical and MRI-based secondary outcome variables were evaluated as well. In designing this study, there was concern about the ethics of performing a placebo-controlled trial in RRMS, because three approved products were readily available. However, a study of brief duration was considered acceptable, provided subjects were fully informed and aware
of the alternatives. A preliminary safety study in MS patients given doses up to 300 mg of oral GA for 10 days revealed only mild adverse events; however, two interim analyses were built into the protocol to permit the data and safety monitoring committee to stop the study in the event of unacceptable toxicity.

Despite the encouraging preclinical data and plausible immunological rationale, this enormous undertaking resulted in a completely negative outcome (J. Wolinsky, personal communication, 2004). After adjusting for baseline characteristics, the mean numbers of relapses were essentially identical for the 50 mg GA, 5 mg GA, and placebo groups. There were no statistical differences in other measurements, including the numbers and proportions of patients who were relapse free, the time to first confirmed relapse, mean change in EDSS scores, or any of the MRI measures. Safety and tolerability were acceptable and did not differ among the three groups. Retrospective subgroup analyses failed to identify any promising results. It is possible, however, that the 50 mg oral dose was sub-therapeutic, and a dose-finding study is under consideration; however, enthusiasm for another oral GA study is lacking in the MS research community.

Clinical Trials of GA in Progressive Forms of MS

Phase II Pilot Study
A study of GA in 106 patients with “chronic progressive” MS (including both secondary progressive and primary progressive forms) was conducted in the mid-1980s (15). Although carefully designed and controlled, this study was marred by problems of insufficient statistical power and inter-site variation. Patients with scores of 2 to 6.5 on the Kurtzke EDSS and a progressive course in the previous two years were followed in a pretrial observation period to confirm progression, then randomized to receive either 15 mg of GA, or a placebo, administered by subcutaneous injection twice daily. The primary endpoint was time to confirmed EDSS progression, maintained for at least three months. Despite the unusual pretrial observation period, stringent progression criteria, and increased dose of GA, its effect on progression was not significant, although all outcome measures showed favorable trends. When data from the two centers were analyzed individually, there was a significant treatment effect at one center, but not the other, which was attributed to failure of placebo-treated patients at that site to progress as expected. Thus, the study tended to support the findings of the relapsing–remitting trials described above, that GA is most effective in early, mild, relapsing MS, and less effective in more disabled patients. However, in a retrospective analysis, the 30 patients in the study with primary progressive MS were found to be divided almost evenly between the GA and placebo groups, and to respond favorably to GA in terms of disability progression after 12 and 24 months of treatment, suggesting that a more extensive trial of GA in patients with primary progressive MS was warranted.

Phase III Trial in Primary Progressive MS (PROMiSe)
Primary progressive disease afflicts 12% to 15% of all MS patients and has a relatively poor prognosis, usually leading to ambulatory disability in 10 years or less. There is relatively little evidence of inflammation on MRI in primary progressive MS, suggesting that an agent such as GA, whose mechanism of action does not primarily involve blood–brain barrier integrity, may be potentially beneficial. In the PROMiSe trial (16), 943 patients (455 males) with progressive spastic paraparesis,
EDSS scores of 3.0 to 6.5, and oligoclonal bands or elevated IgG index in the CSF, were randomized at over 50 centers in the United States, UK, France, and Canada to receive either 20 mg of GA or placebo in a 2:1 ratio by daily subcutaneous injection for a period of three years. The primary efficacy endpoint was time to confirmed disease progression, defined as a change of ≥1 EDSS point for an entry EDSS 3 to 5, or ≥0.5 EDSS point for an entry score of 5.5 to 6.5, sustained for three months. A number of secondary MRI outcome measures, including measurement of brain atrophy, T1 hypointense lesions (“black holes”), gadolinium enhancement, and T2 lesions were assessed, but these data are not yet available.

The study was terminated prematurely after the second interim analysis, based on recommendations of the independent data and safety monitoring committee that could discern no treatment effect on the primary outcome measure, nor project that a treatment effect might occur by the planned end of the trial. The interim analysis was based on 935 subjects with EDSS data, of whom 757 completed at least two years on study or had terminated the study early. Moderately strong trends for delayed time to progression and lower proportion of patients progressing were found for the GA treated group compared with the placebo group; however, these were not statistically significant. Post-hoc analysis showed an effect for males that was not found for females, with the GA and placebo survival curves diverging early, and the difference increasing over time \( P = 0.012 \). Though GA appeared to have some beneficial impact in the male patient cohort, premature discontinuation of the study complicated interpretation of the results (16). The apparent effect on male patients is intriguing, however, as primary progressive MS is known to be more prevalent in males.

**MRI Studies**

*Preliminary Data*

MRI studies were not included in the phase III pivotal trial, except for those done at a single site, which showed a trend toward reduction in enhancing and T2 lesions with GA. In another small study (17), Mancardi and colleagues followed 10 patients with monthly gadolinium-enhanced scans for 9 to 27 months before and 10 to 14 months during treatment with GA. The mean number of new contrast-enhancing lesions was reduced by 57% during treatment. Relapses were also greatly reduced from 2.5 per year in the pretreatment period to 0.3 in the treatment phase of the study. A limited MRI component was belatedly added to the open label extension of the phase III pivotal trial (18). Gadolinium-enhanced and T2-weighted MRI scans were performed in 135 patients, distributed evenly between those originally randomized to GA (mean 6.7 years on treatment) or to placebo (mean 4.0 years on treatment). Surprisingly, significant differences, favoring early initiation of therapy, were found for gadolinium-enhancing lesions, brain atrophy, and a “Z4 composite” score that included those two variables as well as T2 lesion volumes and T1 hypointense lesion volumes. Admittedly, the clinical significance of these findings is problematic in this mixed and selected population followed in the absence of a control group. However, there are no comparable long-term followup data for any other immunomodulator.

**European–Canadian MRI Study in RRMS**

In 1997 a multinational randomized, double-blind, placebo-controlled MRI trial was begun in patients with relapsing–remitting MS and EDSS scores of 0 to 5,
to determine the magnitude and time course of the effect of GA (19). The study population was enriched for patients with active disease by requiring one or more relapses in the two years prior to entry, and one or more contrast-enhancing lesions on baseline MRI scan. A total of 239 eligible patients were randomized to treatment with either placebo or 20 mg/day of GA, but they were followed clinically and radiologically for only nine months. The placebo-treated patients were then crossed over to active drug and all participants were followed for an additional nine months, with scans every three months. Except for the abbreviated duration and inclusion of MRI, the study design was similar to the US pivotal trial, and the patient populations were also comparable in terms of age, disease duration, pre-study relapse rate, and EDSS scores. Treatment and placebo groups were well matched for both clinical and MRI parameters, nearly all patients completed the study, and approximately 95% of the planned MRI scans were available for analysis.

In the double-blind portion of the study, there was a 29% reduction \( (P = 0.003) \) in the primary outcome measure, mean number of gadolinium-enhancing lesions, with GA treatment. Similar results were found for nearly all secondary outcome measures, including new enhancing lesions \( (P < 0.003) \), enhancing lesion volumes \( (P = 0.01) \), new T2 lesions \( (P < 0.003) \), and change in T2 lesion volumes from baseline to month 9 \( (P = 0.006) \). The most striking feature of the study was the time course of the response to GA. Treatment effects for all outcome variables could be expressed as a series of diverging curves with differences between placebo and treatment groups first appearing at three months, and becoming significant at 6 to 7 months. Although enhancing lesions and T2 volumes accumulated in both groups, the rate of accumulation was consistently lower in GA than in placebo-treated patients. In addition, the mean relapse rate was 33% lower in the GA group \( (P = 0.012) \), with nearly all of the difference coming in months 6 to 9. After nine months, 225 of the original 239 patients entered the open-label phase and over 95% completed it, showing that the drug was extremely well tolerated. The effects on MRI were maintained in the group originally treated with active drug, and their relapse rates continued to decline as well. The group that switched from placebo to GA also responded to treatment and developed significantly fewer enhancing lesions \( (P = 0.0001) \) than during the placebo-treatment phase. Similar effects were seen for enhancing lesion volume, change in T2 lesion volume, and relapse rate. Thus, the clinical and MRI benefits of GA treatment found in the placebo-controlled phase were confirmed in the open-label phase of the trial.

However, the effect of GA on MRI measures of disease activity was delayed by 3 to 6 months in contrast to the effect of IFN-β, which is almost immediate (20,21). This may be consistent with the proposed mechanism of action of GA, which involves generation of activated Th2 lymphocytes that cross the blood–brain barrier, become restimulated by myelin antigens within MS lesions, and secrete suppressive cytokines that downregulate the inflammatory autoimmune response (22). As will be described below, this process leads to gradual induction of immune tolerance, but may require several months to take full effect, and is thus highly consistent with the changes seen on MRI. Effects on blood–brain barrier permeability, as detected by gadolinium-enhanced MRI, are probably secondary to reduced disease activity. Furthermore, the MRI effects of GA are consistent with its degree of clinical efficacy, in contrast to the beta interferons which reduce MRI activity by 70% to 90%, but reduce relapse rates or slow progression by only about 30%.
**Effect on Brain Atrophy**

The effect of GA on brain atrophy was evaluated in a post-hoc analysis of scans obtained in the European–Canadian MRI clinical trial (23). Regional brain atrophy was measured using a semiautomated technique based on T1-weighted scans of seven contiguous periventricular slices (24). MRI data were available from 114 placebo-treated patients and 113 GA-treated patients at baseline and at either 9 or 18 months. Average brain volumes were equivalent in the two treatment groups at baseline, and there was no significant difference in the mean rate of volume loss between the placebo and treatment groups during either the double-blind or the open-label phases of the study. The same data were later reanalyzed using the fully automated whole-brain SIENA method to quantify atrophy (25). Using the more precise whole-brain method, Sormani et al. (25) showed that initial treatment with GA for nine months was associated with reduced atrophy progression in the ensuing nine months. Between-group differences during the first nine months were not significant, but the rate of atrophy was slower during the second nine-month period by 0.4% \( (P = 0.015) \), indicating that GA slowed brain atrophy when measured by a more precise technique.

Wolinsky et al. (18) examined GA effects on brain atrophy in 135 relapsing–remitting MS patients in the open-label extension of the pivotal phase III trial, as described above. Sixty nine patients in the extension study originally randomized to GA treatment received GA for a median of 2435 days (6.7 years), while 66 patients randomized to placebo were switched to GA after two years and treated with GA for a median of 1478 days (4 years). Normalized CSF volume was used as a measure of brain atrophy, with an increase in CSF volume indicating increased brain atrophy. Percentage increases in CSF volumes were significantly higher in patients originally randomized to placebo compared with those originally randomized to GA \( (P = 0.041) \), again suggesting a GA treatment effect on brain volume.

**Effect on Black Holes**

New T2 lesions are usually accompanied by gadolinium-enhancing lesions and 65% of these appear hypointense on T1-weighted images. Once enhancement has ceased, approximately 30% of new lesions remain persistently hypointense on postcontrast T1-weighted images. These “black holes” are indicators of axonal loss and permanent tissue disruption, and correlate strongly with MS-related disability (26). Using data from the nine-month double-blind phase of the European–Canadian MRI study, Filippi et al. (27) showed that GA tended to prevent evolution of new gadolinium-enhancing lesions into black holes. A total of 1722 new lesions including 1251 T1 hypointense lesions, 515 (70.7%) in the GA group and 736 (74%) in the placebo group, were detected and followed for a mean of 5.6 months. The percentage of black holes on follow-up scans was lower in GA-treated patients at each time point, and differences became significant seven months after lesion appearance. At month 8, the proportion of new lesions evolving into black holes in the GA group was 15.6% compared with 31.4% in the placebo group \( (P = 0.002) \).

**Adverse Events in Trials and Clinical Experience**

Adverse events in the various GA studies were numerous, but relatively mild, and this has been borne out in clinical practice. There were no hematologic abnormalities, elevated hepatic enzymes, flu-like symptoms, or significant depression. Local
injection site reactions consisting of erythema with or without induration occurred in 90% of GA-treated patients, and were sometimes painful, but never resulted in skin necrosis, although subcutaneous lipatrophy may have been more frequent than with other injectables. An immediate postinjection reaction consisting of variable combinations of flushing, sweating, chest tightness, shortness of breath, palpitations, and anxiety occurred in all of the major studies with frequencies ranging from 15.2% in the US pivotal trial to 37.8% in the European–Canadian MRI trial. The symptoms were sporadic, beginning seconds to minutes after injection, lasting up to 30 minutes, and resolving spontaneously. Most patients had only one or two such reactions over the course of the respective studies, and in practice the reaction has been estimated to occur once in every 1000 to 2000 injections. It does not appear to be allergic in nature, since most patients experience the syndrome only once or twice, have no symptoms after rechallenge with the drug, and have no detectable IgE antibody or other immune markers of allergy to GA. Attempts to study the reaction have been futile because of its unpredictable occurrence and brief duration, and because it cannot be reproduced in animal models. Occasional true allergic reactions with skin rash and urticaria are seen with GA, but they were rare in the reported clinical trials. No adverse effects on pregnancy were reported in any of the clinical trials, and although GA should not be administered in pregnancy, it is classified as a category B drug, which has not been tested in human pregnancy, but is presumed to be safe based on animal studies.

The possible occurrence of neutralizing antibodies to GA is a matter of interest because of the compelling body of evidence that neutralization of IFN-β is important in determining loss of clinical and MRI efficacy (28–30). Nearly all patients receiving GA develop binding antibodies of the IgG1 isotype that can be detected by enzyme linked immunosorbent assay (ELISA) (31). Antibody levels peak at three to four months, then decline to a level slightly above baseline for at least two years of continued treatment. In patients treated for two years, those who were relapse-free at 18 and 24 months of therapy had statistically higher GA antibody titers than treated patients who had one or more relapses, and no correlation between antibody titer and EDSS, or side-effect profile, was observed. Neither polyclonal nor monoclonal GA-specific antibodies interfered with GA activity in vitro (binding to MHC molecules and T-cell stimulation) or in vivo (blocking of EAE) (32). Serum samples from 34 treated patients with GA-specific antibodies were found not to inhibit the proliferative response of GA-specific T-cell clones, nor to interfere with the competitive inhibition by GA of the response to myelin basic protein, nor to inhibit Th2 cytokine secretion. Though contradictory results were reported by Salama et al. (33), their report is marred by inconsistencies. The preponderance of available data suggests that the therapeutic effect of GA is not affected by GA-reactive antibodies, that no evidence of neutralization can be detected, and that no correlations exist between GA antibody titer and the occurrence of relapses or postinjection reactions. Additional long-term studies on GA treated patients may provide further information.

Studies Currently in Progress

Comparative Post-Marketing Studies

There have been several comparative observational studies of GA and the three beta interferons (34–37); in some cases these are prospectively designed, while others are retrospective. Overall, the data from these studies suggest that treatment effects in
reducing relapse rates in observational studies are comparable to those found in randomized clinical trials (38). The studies cited here claim to show that GA is the most effective available therapy, while others suggest that all disease modifying agents are more or less equivalent. All are promoted as representing the “real life” setting of clinical practice, i.e., none of these studies was randomized, and no attempts were made to conduct regular blinded assessments. Therefore, the results should be regarded with caution, if not skepticism, as biases are inevitable. A phase IV multicenter, open label study comparing IFNβ-1a (Rebif®) 44 μg three times per week with GA 20 mg daily is currently being conducted by Serono Inc. Although industry-sponsored, it represents a substantial improvement over the usual observational postmarketing trial, as it is based on a protocol that includes randomization, blinded EDSS examinations, and MRI scans read centrally by radiologists. The primary outcome measure is a comparison of time to first relapse over 96 weeks of treatment, with a secondary objective of comparing the mean number of new or enlarging T2 lesions per subject per scan. Thus, a certain amount of objectivity will be maintained, and the outcome may be more meaningful than the results of most retrospective observational studies.

Combination Studies

**Phase I–II Mitoxantrone Induction.** Clinical and MRI data discussed earlier indicate that the onset of GA efficacy increases gradually over time (19). Long-term open label observations of GA treated patients suggest efficacy may still be increasing even six years after onset of treatment (16). This delay in development of the treatment effect may represent the time needed to shift the immune system from a Th1 to a Th2 bias in GA-treated MS patients. It is possible that the onset of clinical efficacy could be accelerated by using a chemotherapeutic agent to decrease the size of the autoaggressive T-cell pool. Edan et al. (39) showed that early treatment with mitoxantrone, an anthracenedione chemotherapy agent approved for use in MS, reduced relapse rates dramatically, and that the low rates could be maintained by giving maintenance therapy for up to five years. In the current trial, approximately 40 patients with relapsing–remitting MS were randomized to two arms: one with three monthly pulses of mitoxantrone induction therapy followed by GA, and the other with conventional GA treatment alone. Tolerability, safety, clinical efficacy, MRI data, and a number of immunological outcomes will be analyzed over 15 months. The study is conducted and sponsored by Teva Neuroscience.

**CombiRx.** Another study of current interest is the “CombiRx” trial of GA and intramuscular IFNβ-1a in relapsing–remitting MS. The study is based on two conflicting sets of observations: a study in vitro demonstrating additive and synergistic suppression of myelin basic protein (MBP)-specific T-cell lines by combined treatment with IFNβ and GA (40), and a study in vivo showing that combination therapy with GA and IFNα resulted in worsening of EAE in mice (41). Although type I interferons and GA both suppress EAE, they work by different mechanisms. The beneficial effect of IFNβ in MS is partially related to its ability to inhibit matrix metalloproteinase secretion by activated T-cells, reducing their ability to penetrate the blood–brain barrier (42,43). GA activity, on the other hand, probably requires migration of activated T-cells into the CNS, where they produce Th2 cytokines that act via bystander suppression (44,45). Its effects on blood–brain barrier permeability appear to be indirect (46). Thus, treatment with IFNβ could theoretically impede access of GA-activated cells to sites of inflammation in the CNS. A small 12-month study (47) was conducted...
to assess the effect of combination therapy with IFNβ-1a 30 μg/wk i.m. (Avonex) and GA on MRI scans and clinical outcomes in 31 relapsing–remitting MS patients who had been taking IFNβ-1a for at least 6 months. The combination was found to be safe, and enhancing lesions were significantly reduced from baseline to 12 months, suggesting increased effectiveness with combination therapy. At one participating center, GA-reactive T-cell lines were isolated from patients on combined therapy and on GA monotherapy. There was no difference in the proportion of T-cell clones of the Th2 phenotype (48). The result suggests that IFNβ-1a does not interfere with the immunological response to GA, nor with the ability of GA-reactive cells to enter the CNS. A definitive phase III CombiRx trial of IFNβ-1a and GA is currently being conducted, independent of pharmaceutical support, with funding from the National Institutes of Health.

**IMMUNOLOGICAL ACTIVITY OF GA**

**Studies in EAE**

In the past few years, a wealth of new information has appeared on the remarkable immunological and neurobiological properties of GA, some of which may account for its long-term therapeutic efficacy in MS. GA is a synthetic mixture of polypeptides composed of four amino acids, L-alanine, L-glutamic acid, L-lysine, and L-tyrosine, synthesized in the 1960s at the Weizmann Institute of Science in Israel as one of several peptides designed to resemble myelin basic protein (MBP) (49). These copolymers were originally used to investigate the interaction of myelin proteins and lipids thought to be responsible for induction of EAE, which can be induced by sensitization with encephalitogenic peptide fragments of the MBP molecule, and prevented or modified by many antigen-specific and nonspecific manipulations, including treatment with nonencephalitogenic fragments of MBP (50). When the copolymers were initially tested, they failed to induce EAE, but the animals were protected when rechallenged with MBP (51). GA (known as copolymer-l or cop-1) was the most effective of these substances (52), and was therefore selected for further investigation. Perhaps the most intriguing observations from the point of view of MS therapy were those on chronic relapsing EAE, which closely mimics the human disease in terms of clinical activity and pathology (53). Pretreatment with GA reduced or delayed clinical relapses, and administration of GA after onset of disease modified the duration and intensity of relapses. These early observations showed that GA could suppress an ongoing autoimmune response after establishment of disease, an obvious requirement for treatment of MS. GA was also remarkably nontoxic, producing no significant adverse reactions in treated animals. Moreover, it did not appear to interfere with systemic immunity to non-neural antigens.

**Proposed Mechanisms of Action**

*Interference with T-Cell Activation*

Early studies of cellular and humoral immune responses indicated that GA has at least partial cross-reactivity with MBP, and reacts with monoclonal antibodies raised against MBP, as well as with T-cells or T-cell lines sensitized to MBP (54–57). Other investigators, however, have disputed the claims that GA and MBP are antigenically cross-reactive in the strict sense (58,59). Teitelbaum and coworkers reported that GA
could specifically inhibit proliferation and IL-2 secretion by murine and human MBP-specific T-cell lines and clones in vitro, through competition with MBP for binding to MHC class II molecules on antigen-presenting cells. More recently, direct “promiscuous” binding of GA to human antigen-presenting cells and purified HLA-DR molecules was shown (60,61). Furthermore, GA can inhibit binding of MBP or the MBP peptide p84–102 to these cells through competition for MHC class II surface molecules. Thus, it may be more appropriate to characterize GA and MBP as mutually inhibitory, rather than cross-reactive.

The relative specificity of GA for MBP may seem paradoxical in view of its random molecular structure and striking lack of specificity for species, MBP epitope, or MHC restriction. Alternatively, the specificity of GA for MBP may be a function of limited testing, as suggested by Racke et al. (62), who found that GA inhibited in vitro responses of T-cell hybridomas specific for ovalbumin and insulin. Furthermore, GA can inhibit binding of myelin proteolipid protein (PLP) and myelin-oligodendrocyte glycoprotein (MOG), both of which are encephalitogenic, to MHC molecules on antigen-presenting cells (63), and can suppress EAE induced in mice by PLP (64) and MOG (65). Since there is essentially no sequence homology between MBP and PLP or MOG, the suppressive effect may be attributed to induction of Th2 cytokines and bystander suppression.

**Immune Deviation from Th1 to Th2 Phenotype**

GA induces proliferation of naive normal T-cells, and GA-reactive T-cell lines can easily be generated from normal individuals (40,57). These observations were recently confirmed by Hafler et al. (66), who characterized GA as a “universal antigen” that induces proliferation in T-cell lines from normal or MS subjects, independent of any prior exposure. In MS patients treated with GA, the proliferative response declined gradually, and after several months could not be restored with IL-2, consistent with the development of tolerance by activation-induced cell death (67). GA-reactive T-cell lines secreted the suppressive cytokines IL-5 and IL-13. They were also shown to cross-react with a combinatorial peptide library based on the immunodominant epitope of MBP, indicating “degeneracy” of the T-cell receptor, and suggesting that GA may act as an altered peptide ligand to induce Th2 cells in response to itself or to MBP peptides. Evidence for suppressor activity of GA in vivo has evolved rapidly since the Th1/Th2 paradigm of T-cell regulation has become widely appreciated. The early observation that spleen cells from mice treated with GA could transfer protection against EAE suggested that protection was mediated by regulatory cells (68). This was later confirmed by showing that T-cell lines induced with GA could inhibit the response of MBP-specific T-cell lines and prevent active induction of EAE (69). Subsequently, a number of additional studies were reported supporting the finding that GA induces and activates CD4+ cells of the Th2/Th3 phenotype that secrete the suppressive cytokines IL-4, IL-6, IL-10, and TGFβ, but not the proinflammatory Th1 cytokines IFNγ or TNFα (44,45).

Treatment of MS patients with GA for one year was reported (22) to upregulate IL-4 and TGFβ, reduce expression of TNFα, and increase serum levels of IL-10. The most striking feature of these findings was their slow development over three to six months, which corresponds to the delay in generation of MBP cross-reactive Th2 cells seen in vitro (45), and to the delay in clinical and MRI efficacy noted in the recent European/Canadian MRI study (19). Furthermore, treatment with GA results in increased apoptosis of a substantial percentage of activated CD4+ T-cells.
As the shift from a Th1 to a Th2 state is established, the number of Th1 GA-reactive cells decreases, leading to reduced entry into the CNS over time (70). Chen et al. (71) showed that the Th2-biased response with GA is sustained over long-term treatment. Proliferative responses, cytokine production and cross reactivity with whole MBP and the MBP p83–99 peptide were compared between 10 relapsing–remitting MS patients who had been on GA for six to nine years and 10 patients beginning treatment. Long-term treatment with GA resulted in a 2.9-fold decrease in the estimated precursor frequency of GA-reactive T-cells. Nevertheless, the sustained response to GA remained Th2-biased and partially cross-reactive with MBP and MBP peptide. These findings are consistent with the proposed mechanism of action of the drug; however, the cytokine study was confined to a small number of patients, and has not yet been confirmed. Thus, the mechanism of action of GA in humans remains somewhat hypothetical, although evidence for real and reproducible regulatory effects on the immune system is clear.

**Bystander Suppression Within the CNS**

GA treatment stimulates GA-reactive T-cells, which progressively assume Th2 characteristics (72). In vitro models demonstrate that Th2 cells can penetrate the CNS more easily than Th1 cells (73). The detection of GA-reactive T-cells in the CNS of EAE mice (72,74) led to the hypothesis that similar events occur in treated MS patients. GA-reactive Th2 cells in the CNS are hypothesized to decrease local inflammation through “bystander suppression”. As GA is rapidly metabolized in subcutaneous tissue at the site of administration, the antigen presented within the CNS cannot be GA, and must be endogenously derived. It is postulated that the GA-specific Th2 cells within the CNS are restimulated by products of myelin turnover presented by local APCs. Local reactivation of GA-specific T-cells triggers the release of anti-inflammatory cytokines such as IL-4, IL-6, IL-10, TGFβ and brain-derived neurotrophic factor (BDNF), but not IFNγ (74,75). The production of proinflammatory cytokines, including IL-2 and IFNγ, is inhibited through the bystander effect. Recently an interesting report (76) comparing Th1 and Th2 lymphocyte migration across a model blood–brain barrier system showed that IFNβ inhibited Th1 cells from entering the CNS, while GA increased the migration of Th2 cells. If this paradigm holds true in vivo, it may provide a convincing rationale for combined therapy with IFNβ and GA as proposed in the CombiRx study described earlier.

**Restoration of Defective Immune Regulation**

In addition to driving CD4+ T-cells toward the Th2 phenotype, GA may exert effects on CD8+ T-cells, which are reduced in patients with MS, compared to controls (77). Treatment with GA results in upregulation of the CD8+ response with restoration to levels observed in healthy individuals. Although IFNγ and TNFα are produced by GA-specific CD8+ T-cells, they could regulate autoimmune responses systemically in lymphoid tissue or centrally in the nervous system (78). Clearly, further studies are required on the effects of GA on the various T-cell populations in patients with MS.

**Neuroprotection**

Experimental evidence in animals suggests that inflammatory cells may have a dual role in tissue damage and protection, termed protective autoimmunity (79–81).
The concept is controversial, and protection in one area of the nervous system may be followed by injury elsewhere when animals immunized with MBP or treated with myelin-reactive T-cells develop EAE. However, in animal models of optic nerve crush injury (82) or intraocular glutamate injection (83), passive transfer of GA-reactive T-cells protected retinal neurons, without subsequent development of EAE. The neuroprotective effects of GA have since been demonstrated in several other animal models. For example, GA treatment reduced axonal damage in mice with chronic EAE (84), and increased survival time and improved motor function in a murine model of amyotrophic lateral sclerosis (85). Another recent study (86) demonstrated that adoptive transfer of GA-reactive T-cells could protect mice against MPTP-induced degeneration of nigrostriatal neurons, suggesting a novel treatment for Parkinson’s disease.

To date, however, the mechanism of protective autoimmunity, or of GA-mediated protection, remains uncertain. Nitric oxide has been implicated in the pathogenesis of MS (87), and Kayhan et al. (88) demonstrated that GA treatment of EAE in mice caused a significant decrease in nitric oxide secretion by splenic lymphocytes, potentially preventing the demyelination caused by this inflammatory mediator through its action on oligodendrocytes or myelinated axons. A more likely possibility involves BDNF, which plays an important role in survival and differentiation of neurons, and has been demonstrated in inflammatory brain lesions including MS plaques (89). Ziemssen et al. (90) reported that GA-specific T-cell lines of either the Th1 or Th2 phenotype could produce BDNF, and Chen et al. (75) showed that BDNF production was higher for GA-reactive T-cell lines than for MBP or tetanus toxoid-reactive lines, although only a small percentage of GA-reactive lines secreted significant levels of BDNF. Under the proper conditions, BDNF secreted by GA reactive Th1 and Th2 cells in the CNS may exert direct neurotrophic effects within MS plaques, and could perhaps account for observations such as the reduction in evolution of enhancing MRI lesions into “black holes” (27). However, the mechanism of neuroprotection in MS, even by GA-reactive Th2 cells, is still not clearly understood, and is the topic of intense ongoing investigation.

Pharmacogenomic Studies

In the future, clinical indicators of successful therapy with interferons or GA may become available. Fusco et al. (91) performed a retrospective analysis of 44 MS patients treated with GA and 29 treated with weekly injections of IFNβ-1a who were typed for MHC genes. Both groups showed the expected reduction in relapses on treatment compared with the two prior years. The MHC haplotype of the subjects had no influence on the apparent clinical response for those on IFNβ-1a; however, there was a positive correlation between presence of DRB1*1501 and response to GA therapy ($P = 0.008$). The results, if confirmed in larger studies, suggest that host genetic factors may determine the selection of either GA or interferons in relapsing–remitting MS. In another study, Farina et al. (92) showed that selected MS patients who were nonresponders to GA differed in their immune response profile to GA when compared with a cohort of clinical responders. Though the finding is preliminary and needs to be confirmed, it suggests that a combination of genetic background, early immune response to the drug, and MRI measures could perhaps be used together as predictors of GA and interferon long-term treatment outcomes. The mechanism of action of GA has been intensively studied in the past few years, as summarized in several excellent reviews (93–96), to which the reader is referred.
The immunologic properties of GA are partially understood, although many details remain to be clarified, and may be briefly summarized as follows: GA is a universal antigen that binds readily to multiple MHC types in both normal individuals and MS patients, induces proliferation, and acts as an altered peptide ligand to activate antigen-specific CD4\(^+\) T-cells, polarizing them toward the Th2 phenotype. These activated cells cross the relatively intact blood–brain barrier, react with epitopes of MBP, and secrete regulatory cytokines such as IL-4, IL-10, IL-13, and TGF\(\beta\), which downregulate the synthesis of proinflammatory cytokines within active MS plaques, leading to bystander suppression of the ongoing autoimmune response to multiple myelin antigens including PLP and MOG, and ultimately to amelioration of the inflammatory, demyelinating, and neurodegenerative process. At the same time, the GA-reactive cells mediate a neuroprotective response that may nonspecifically affect not only myelin, but also neurons and axons.

THE PLACE OF GA IN MS THERAPY

Comparisons between GA and the beta interferons are potentially hazardous because each agent was tested in separate studies, under different conditions, and in patient populations with different pre-study demographics, relapse rates, and levels of disability. IFN\(\beta\)-1b was originally shown to have no convincing clinical effect on progression of MS, even though the trial was continued for up to five years in a subgroup of patients (97,98). Nevertheless, its effect on relapse rate and prevention of new MRI lesions was dramatic (99), suggesting that slowing of sustained neurological impairment might ultimately be expected. This was recently confirmed in the European study of IFN\(\beta\)-1b in secondary progressive MS (100), although a similar North American study failed to show an effect on progression (101,102). IFN\(\beta\)-1a (Avonex) was reported to slow progression (103), although tested in a much shorter clinical trial and in a population with early relapsing–remitting disease, raising questions as to whether one-point changes at the lowest levels of the EDSS reflect genuine disability. Studies of IFN\(\beta\)-1a (Rebif) have resulted in highly significant effects on relapses, MRI activity, and progression in relapsing–remitting MS (104), but no significant therapeutic effect on disability progression in secondary progressive MS (105). GA was shown to have a significant effect on relapses and disability scores in a patient population with mild to moderate disability, and a modest effect on MRI activity that was consistent with its clinical efficacy. Any attempt to compare GA with the various IFN\(\beta\) products is subject to bias, as shown by the open-label comparative studies that add little to our knowledge of these therapies, though they appear to be useful marketing tools.

In the opinion of most neurologists, prevention or retardation of disability is the gold standard for MS treatment; however, agents that reduce the relapse rate may have substantial long-term benefit as well. The episodes of incapacity caused by MS attacks often result in time lost from work or other activities of daily living, increased medical expenses, and emotional distress. Thus, agents that prevent even one-third of relapses, providing they are easily administered and free of toxicity, represent substantial progress in the management of MS. Furthermore, prevention of acute attacks and of subclinical disease activity may have a long-term beneficial effect on the risk of future disability, as implied by epidemiological studies (106) and reports of frequent MRI scanning of MS patients (107–109). It is likely that this will prove to be true for all of the currently available agents.
A major advantage of GA over IFNβ-1b, and to a lesser extent IFNβ-1a, is its favorable side effect profile consisting of relatively mild injection site reactions and the uncommon systemic postinjection reaction that immediately follows a small percentage of injections. Nearly all patients who experience such a reaction quickly realize that it is not hazardous, and accept the possibility of another one as a minor inconvenience. In contrast, patients beginning treatment with IFNβ are subject to injection site reactions, an annoying flu-like syndrome that often (but not always) diminishes over time, and a variety of other less common symptoms, including the worrisome risk of severe depression. In addition, 20% to 30% of patients taking high dose IFNβ (either Betaseron or Rebif) for 18 to 24 months develop neutralizing antibodies, and may cease to respond to treatment. The frequency of neutralizing antibodies to IFNβ-1a (Avonex) is considerably lower (approximately 5%), but at the expense of reduced efficacy (110). GA, in contrast, induces binding antibodies in all or nearly all patients, but thus far no evidence of neutralizing activity has been detected. In fact, its efficacy seems to increase with prolonged treatment. Finally, although none of these agents should be taken during pregnancy, the risk appears to be least with GA.

CONCLUSION

GA is an unique noninterferon, nonsteroidal therapy for MS that may be considered at least partially immunospecific. It has been extensively studied, and its mechanism of action is relatively well understood in the light of current immunological concepts. Not only has it been shown, in randomized controlled clinical trials, to be at least as effective as the beta interferons, but also its efficacy appears to increase with time. Furthermore, it has the most favorable side effect profile of all agents available to treat MS. Therefore, it should be considered as first-line therapy for ambulatory patients with clinically definite relapsing–remitting MS, and treatment should be started as soon as possible after the diagnosis is established. In addition, it is suitable as alternative therapy for patients treated with IFNβ who are unable to tolerate the drug, or who are able to take it only at reduced dosage because of persistent side effects, and in patients treated successfully with IFNβ who, after a period of time, lose efficacy because of the development of neutralizing antibodies. In the future, GA may prove to be useful in combination with interferons or other drugs. These ongoing studies, as well as the clinical experience of neurologists and patients, will help to define further the role of this novel and increasingly important therapeutic agent.

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Mitoxantrone

Oliver Neuhaus, Bernd C. Kieseier, and Hans-Peter Hartung
Department of Neurology, Heinrich Heine University, Düsseldorf, Germany

INTRODUCTION

Mitoxantrone (Fig. 1) was developed in the 1970s and is an antineoplastic agent. It is an anthracenedione derivative related to the anthracyclins doxorubicine and daunorubicine. It interacts with topoisomerase-2, stabilizes its cleavable complex with DNA, thus prevents the ligation of DNA strands, and consequently delays the cell-cycle progression (1). Mitoxantrone is used to effectively treat malignancies such as breast and advanced prostate cancer, lymphoma, and leukemia (2). Furthermore, in common with other antineoplastic agents, strong immunosuppressive properties of mitoxantrone have been observed providing a rationale for its use in autoimmune disorders (3–6).

EVIDENCE LEADING TO THE APPROVAL OF MITOXANTRONE FOR USE IN MULTIPLE SCLEROSIS

Mitoxantrone in Experimental Autoimmune Encephalomyelitis

In the 1980s, mitoxantrone was proven effective in both actively and passively induced experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS) (7–9). Ridge et al. (7) observed a dose-dependent inhibitory effect as determined by clinical evaluation and histopathology in rat EAE. Interestingly, mitoxantrone was 10 to 20 times more effective than cyclophosphamide in suppressing the development of EAE. Moreover, in an adoptive transfer model, encephalitogenic T-cells treated with mitoxantrone, prior to injection, were unable to induce the disease, indicating an inhibitory effect of mitoxantrone on T-cells. Clinical relapses were prevented or ameliorated (8,9).

Clinical Studies of Mitoxantrone in MS

In a number of small open trials, positive effects of mitoxantrone in MS were shown (Table 1) (4).
In a randomized, magnetic resonance imaging (MRI)–controlled, but clinically unblinded and not placebo-controlled, trial in France and the United Kingdom, the effects of mitoxantrone were assessed in patients with very active MS, defined as frequent severe relapses without clinical remittance (14). Forty-two patients were randomized and received monthly infusions of either 20 mg mitoxantrone (irrespective of the body surface) plus 1 g methylprednisolone (MP) or 1 g MP alone for six months. The primary endpoint was the percentage of patients without new active MRI lesions. At study entry the percentages were: mitoxantrone plus MP, 10%; MP alone, 4.8%. After six months, the numbers were: mitoxantrone plus MP, 90%; MP alone, 31% ($P < 0.001$).

In an Italian trial, the efficacy of mitoxantrone was assessed in 51 patients with relapsing–remitting (RR) MS (15). Inclusion criteria were an expanded disability status scale (EDSS) between two and five (16) and at least two relapses within the previous two years. The patients were randomized and received either mitoxantrone (8 mg/m$^2$ body surface) or placebo. Clinical assessment was performed by blinded physicians. The primary endpoint was the percentage of patients with clinical progression, defined as an EDSS increase by one point. After 24 months of observation, 9 out of 24 patients with placebo (37%) and 2 out of 27 patients with mitoxantrone (7%) deteriorated clinically by one EDSS point ($P = 0.02$). Regarding the secondary endpoints, mitoxantrone was partially superior to placebo.

A comparative double-blind trial of mitoxantrone (13 infusions of 12 mg/m$^2$ body surface) versus MP (13 infusions of 1 g, both groups over 32 months) in 49 patients with secondary progressive (SP) MS, performed in Belgium, revealed a significant improvement of the EDSS and a significant decrease of the total number of gadolinium-enhancing lesions in the mitoxantrone group (17).

**Table 1** First Clinical Studies of Mitoxantrone in Multiple Sclerosis

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of patients</th>
<th>MS course</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mauch et al. (10)</td>
<td>6</td>
<td>Relapsing</td>
<td>12 mg/m$^2$ every 3 mo</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Progressive</td>
<td></td>
</tr>
<tr>
<td>Gonsette and Demonty (11)</td>
<td>16</td>
<td>Relapsing</td>
<td>14 mg/m$^2$ body surface</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>every 3 wk 3 cycles followed by 14 mg/m$^2$ every 3 mo for up to 2 yr</td>
</tr>
<tr>
<td>Kappos et al. (12)</td>
<td>14</td>
<td>Rapidly progressing</td>
<td>10 mg/m$^2$ every 3 wk</td>
</tr>
<tr>
<td>Noseworthy et al. (13)</td>
<td>13</td>
<td>Progressive</td>
<td>8 mg/m$^2$ every 3 wk</td>
</tr>
</tbody>
</table>

*Abbreviation: MS, multiple sclerosis.*
The largest phase III study, thus far, is the mitoxantrone in multiple sclerosis (MIMS) study, which led to approval for the treatment of MS number of regulatory authorities (18). The MIMS study was a randomized, placebo-controlled, investigator-blinded multicenter trial in patients with worsening RRMS and SPMS. One hundred and ninety four patients with an EDSS between three and six were randomized and divided into three groups: (i) mitoxantrone 12 mg/m² body surface, (ii) mitoxantrone 5 mg/m², and (iii) placebo (methylene blue). All patients received mitoxantrone or placebo intravenously every three months for two years. The primary study endpoint was a multivariate analysis of five different clinical parameters (change from three neurological baseline scores including EDSS after 24 months, time for first treated relapse, and number of relapses treated with steroids). After two years, 188 patients still participated in the study. In all five parameters, the mitoxantrone 12 mg/m² group was significantly superior compared to the other groups. Progression of disability and relapse rate were significantly reduced. This therapeutical effect was still measurable even after 12 months of final infusion. In a subgroup of 110 patients, in addition to the clinical investigation, MRI assessment was performed and analyzed in a central laboratory (19). Significantly fewer patients receiving 12 mg/m² mitoxantrone demonstrated enhancing lesions at 24 months relative to placebo (0% vs. 15.6%, \( P = 0.02 \)). The mean increase in the number of T2-weighted lesions was 0.29 in 12 mg/m² mitoxantrone and 1.94 in placebo recipients \( (P = 0.03) \). In both mitoxantrone groups, a significant reduction of new lesions and a reduced burden of disease were observed. Table 2 summarizes the most important aspects of the controlled clinical trials.

### Table 2  Mitoxantrone in Multiple Sclerosis–Controlled Clinical Trials

<table>
<thead>
<tr>
<th></th>
<th>French–British trial (14)</th>
<th>Italian trial (15)</th>
<th>MIMS trial (18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>42</td>
<td>51</td>
<td>194</td>
</tr>
<tr>
<td>Clinical course of MS</td>
<td>Active RRMS/SPMS</td>
<td>RRMS</td>
<td>Active RRMS/SPMS</td>
</tr>
<tr>
<td>Dosage</td>
<td>Mitoxantrone 20 mg (absolute dose)+ MP 1 g</td>
<td>8 mg/m² body surface</td>
<td>5 vs. 12 mg/m² body surface</td>
</tr>
<tr>
<td>Treatment frequency</td>
<td>Monthly</td>
<td>Monthly</td>
<td>Every 3 mo</td>
</tr>
<tr>
<td>Treatment duration</td>
<td>6 mo</td>
<td>12 mo</td>
<td>24 mo</td>
</tr>
<tr>
<td>Observation duration</td>
<td>6 mo</td>
<td>24 mo</td>
<td>24 mo</td>
</tr>
<tr>
<td>Reduction in progression of disability (^a) (%)</td>
<td>84</td>
<td>79</td>
<td>64</td>
</tr>
<tr>
<td>Reduction of relapse rate (%)</td>
<td>77</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Reduction of new MRI lesions (%)</td>
<td>84</td>
<td>52</td>
<td>85</td>
</tr>
<tr>
<td>Reduction of active MRI lesions (%)</td>
<td>86</td>
<td>n.d.</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^a\)Deterioration by one EDSS point.

**Abbreviations:** EDSS, expanded disability status scale; MIMS, mitoxantrone in multiple sclerosis; MP, methylprednisolone; MRI, magnetic resonance imaging; MS, multiple sclerosis; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; n.d., not determined.
CURRENT CLINICAL ASPECTS OF MITOXANTRONE

Indication—Which Patients Should Be Treated?

Several national and international medical advisory boards to MS societies recommend the use of mitoxantrone in patients with RRMS who have frequent relapses and incomplete remissions and those with SPMS having rapid progression (by at least one EDSS point per year). Decision to use the treatment should be given by experienced neurologists at clinical MS centers. Although the following treatment recommendations reflect expert opinions, have no definite evidence base, and three groups of MS patients are recommended for treatment with mitoxantrone (18,20,21):

1. RRMS patients with two or more relapses per year, incomplete remission (EDSS ≥3) and insufficient response to IFN-β or glatiramer acetate;
2. SPMS patients with marked progression of disability (≥1 EDSS point per year) and/or high relapse rate (≥2 relapses per year); and
3. SPMS patients with rapid progression (≥1 EDSS point per year) without relapses.

According to the primary endpoints of the different clinical studies with mitoxantrone, the therapeutic goal is the clinical and MRI stabilization of the disease. The characterization criteria of responders or nonresponders and the treatment duration before the evaluation of a clinical response are not yet defined. A suggested, but not evidence-based, marker to detect a clinical nonresponder is the deterioration of one EDSS point after one year of treatment.

For primary progressive (PP) MS patients treatment options are limited. For mitoxantrone, efficacy in PPMS is currently addressed in clinical trials (22), although the results presented thus far are disappointing (23).

Dosage and Duration of Treatment

According to regularly body approvals based on the clinical trials, the currently recommended mitoxantrone dose is 12 mg/m² body surface administered intravenously every three months. In some countries, the dosage regimen of the French-British trial is approved, i.e., 20 mg monthly for six months, irrespective of the body surface (6,14). Patients with aggressive MS can be considered for treatment with an induction therapy with mitoxantrone 10 to 12 mg/m² monthly for the first three months followed by the regular trimester scheme (20). The optimal dosage regimen remains to be evaluated and is currently being assessed in a European clinical trial comparing three different doses (5, 9, and 12 mg/m² body surface) (6).

Practical Guidelines

Before onset of therapy, a number of laboratory exams are recommended including a pregnancy test (during pregnancy and nursing, mitoxantrone is contraindicated), chest X-ray, electrocardiography, and echocardiography with quantitative assessment of the left ventricular ejection fraction (LVEF). Due to the risk of infertility, male patients should be offered the opportunity to cryopreserve their sperms. According to the lab results (white blood count, thrombocytes, and liver enzymes), dose adjustments should be performed as shown in Table 3. Antiemetic protection in parallel to the mitoxantrone infusion may be helpful. The most clinically relevant interactions of mitoxantrone are with phenytoin (decreased plasma concentration)
and angiotensin converting enzyme inhibitors (increased bone marrow toxicity). Due to the increased risk of infections, patients treated with mitoxantrone should not receive live vaccines. Patients with hepatic disturbances require reduced doses of mitoxantrone (Table 3), whereas for patients with renal disturbances there are no restrictions.

Safety and Tolerability

The MIMS trial and preceding studies exhibited a generally good safety profile of mitoxantrone (Table 4) (18). Adverse events were rare and mild to moderate. However, long-term follow-up data are still pending for finally evaluating the safety profile of mitoxantrone. To this end, a large open-label multicenter study of mitoxantrone in 509 MS patients with a five-year observation period and a broad number of outcome measures (Registry to Evaluate Novantrone® Effects in Worsening MS) is currently underway (24).

Cardiotoxicity

Treatment with mitoxantrone is restricted to a cumulative total life dose of 140 mg/m² body surface, i.e., when using the standard dose of 12 mg/m², the treatment must be discontinued after approximately 12 infusions. The reason for this restriction is the increased risk of an irreversible congestive cardiomyopathy beyond the threshold of 140 mg/m² body surface as observed in cancer patients treated with mitoxantrone (25,26). A recently published retrospective study has investigated the risk of mitoxantrone-induced cardiotoxicity in patients with MS (27). In this study, data obtained from 1378 patients from three clinical trials were analyzed: the MIMS trial (124 patients) (18), a French open multi-center trial (802 patients) (28,29), and a

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<table>
<thead>
<tr>
<th>Event</th>
<th>Recommended dose (mg/m² body surface)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC 1.0–1.99 T/1 and/or thrombocytes 25–49 T/1 3 wk after last infusion</td>
<td>10</td>
</tr>
<tr>
<td>WBC &lt; 1.0 T/1 and/or thrombocytes &lt; 25 T/1 3 wk after last infusion</td>
<td>8</td>
</tr>
<tr>
<td>WBC 3.0–3.99 T/1 and/or thrombocytes 75–99 T/1 1 wk before infusion</td>
<td>9</td>
</tr>
<tr>
<td>WBC 2.0–2.99 T/1 and/or thrombocytes 50–74 T/1 1 wk before infusion</td>
<td>6</td>
</tr>
<tr>
<td>WBC &lt; 2.0 T/l and/or thrombocytes &lt; 50 T/l 1 wk before infusion</td>
<td>No infusion</td>
</tr>
<tr>
<td>Non-hematological toxicity (WHO grade 2 or 3)a</td>
<td>10</td>
</tr>
<tr>
<td>Non-hematological toxicity (WHO grade 4)</td>
<td>No infusion</td>
</tr>
</tbody>
</table>

aNausea: WHO grade 2 = transient nausea; WHO grade 3 = nausea requiring therapy. Liver enzymes: WHO grade 2 = 2.6–5.0 x upper reference value; WHO grade 3 = 5.1–10.0 x upper reference value. Alopecia: WHO grade 2 = moderate alopecia, alopecia areata; WHO grade 3 = complete but reversible alopecia. Abbreviations: WBC, white blood count; WHO, World Health Organization. Source: From Ref. 18.
retrospective German trial (452 patients) (30). The mean treatment duration was 29 months; the mean cumulative dose was 61 mg/m² body surface. One hundred and forty one patients had received a cumulative mitoxantrone dose of more than 100 mg/m² body surface. Two of the 1378 patients developed a lethal congestive heart failure after onset of therapy with mitoxantrone. One of the two patients had received a cumulative dose of 162 mg/m² body surface. The other patient had received only one single dose of 9 mg/m²; after one year, her LVEF was <50%; four years after treatment with mitoxantrone, she died of congestive heart failure, the relationship of which to the previous mitoxantrone therapy remains uncertain. Seven hundred and seventy-nine patients examined by echocardiography before and during treatment. In 17 of these 779 patients, a reduction of the LVEF below 50% was observed. All 17 patients had received a cumulative dose of more than 100 mg/m². More recently, one more mitoxantrone-treated MS patient with congestive heart failure was documented in a case report (31).

The pathomechanisms of the mitoxantrone-associated cardiotoxicity remain elusive. Proposed mechanisms are based on (i) free radicals (32), (ii) oxidative stress (33), (iii) altered function of myocardial adrenergic receptors (34), (iv) disturbed calcium transport in the cardiac sarcolemma (35), (v) lipid peroxidation (36), and (vi) cytokines such as tumor necrosis factor (TNF)-α or interleukin (IL)-2 (37).

Currently, several strategies are being pursued to circumvent the problem of mitoxantrone-associated cardiotoxicity. These include giving pulses of reduced doses

<table>
<thead>
<tr>
<th>Frequency (%)</th>
<th>Mitoxantrone</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>76a</td>
<td>20</td>
</tr>
<tr>
<td>Alopecia</td>
<td>61a</td>
<td>31</td>
</tr>
<tr>
<td>Menstrual disorder</td>
<td>61a</td>
<td>26</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>53</td>
<td>52</td>
</tr>
<tr>
<td>Urinary tract infections</td>
<td>32a</td>
<td>13</td>
</tr>
<tr>
<td>Secondary amenorrhea</td>
<td>25a</td>
<td>0</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>19a</td>
<td>0</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Increased liver enzymes</td>
<td>15a</td>
<td>3</td>
</tr>
<tr>
<td>Urine abnormal</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Electrocardiography abnormal</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Constipation</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Rhinitis</td>
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<td>14</td>
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<tr>
<td>Pharyngitis</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Sinusitis</td>
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<td>2</td>
</tr>
<tr>
<td>Viral infection</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Headache</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Anemia</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

*aSignificantly more common in active drug recipients than in placebo group (P < 0.05).

Source: From Ref. 18.
of mitoxantrone in order to prolong its application. Furthermore, animal data has revealed that the combination of mitoxantrone with the cardioprotector dexrazoxane may be useful to ameliorate or even prevent the mitoxantrone-associated cardiotoxicity (38–40). Interestingly, in a recent publication, dexrazoxane was shown to increase the efficacy of mitoxantrone in EAE (40). An alternative would be the development of other anthracenedione derivatives with lower cardiotoxicity (41).

**Therapy-Related Acute Leukemia**

Mitoxantrone and other topoisomerase-2 inhibitors have been reported to induce acute leukemia. In the retrospective study with 1392 MS patients mentioned above, one case was observed (0.07%) (42). Other seven case reports have been published (43–49). Retrospectively, Voltz et al. (49) calculated a relative risk of 0.21%.

**Other Adverse Events**

Mitoxantrone is generally well tolerated. For further adverse events documented in the MIMS trial, see Table 4. Secondary amenorrhea occurs in up to 10% of female patients treated with mitoxantrone (29). Paravasation of the compound has to be strictly avoided as tissue damage may occur. In case of accidental paravasation, the infusion should be immediately interrupted, and the patient should receive steroids (hydrocortisone, 100 mg intravenously and 100 mg fractionated subcutaneously into the paravasal space).

**PUTATIVE MECHANISMS OF ACTION OF MITOXANTRONE**

Apart from the cytotoxic efficacy of mitoxantrone, immunosuppressive effects and even antiviral and antibiotic effects have been observed. More recently, immunomodulatory properties have been suggested, as a number of distinct immunological effects have been described (3,50,51). Possible action sites of mitoxantrone in the putative pathogenesis MS (see Chapter 4) are shown in Figure 2. Still, more research is warranted to access the immunological effects of mitoxantrone in MS, as its specific mechanisms of action in targeting the immune system still remain unclear.

**Immunosuppressive Properties**

As well-established for decades, mitoxantrone is a potent immunosuppressive agent targeting proliferating immune cells (10,53–55). It inhibits proliferation of macrophages, B-lymphocytes, and T-lymphocytes (53,10).

**Effects on Helper and Suppressor T-Cells**

In an in vitro system testing an anti-sheep red blood cell response, mitoxantrone was observed to inhibit T helper activity and, conversely, to enhance T suppressor functions (54). In contrast, in an in vivo mouse model, the induction of suppressor T-cells was also abrogated by mitoxantrone (54). In addition, T helper cells were indirectly inhibited by mitoxantrone-induced macrophages.
Mitoxantrone has been shown to induce apoptosis of B-lymphocytes (56) and other types of antigen-presenting cells (57). Comparison of peripheral blood mononuclear cells (PBMC) obtained from MS patients before and immediately after application of mitoxantrone exhibited a decreased proliferation of PBMC based on necrotic cell death, predominantly in B-cells (58). Thus, there may be a bimodal mechanism of cell death induced by mitoxantrone: apoptosis at lower concentrations and cell lysis at higher concentrations. Previous pharmacokinetical studies in oncology revealed maximum serum concentrations of mitoxantrone between 308 and 839 ng/mL and terminal half-lifes between 38.4 and 71.5 hours (59–62). Thus, in the first approximately 10 days after infusion, maximum serum concentrations are higher than 20 ng/mL [a putative threshold between induction of necrosis and apoptosis (57)], whereas the following time after infusion (approximately 80 days at a three-month dosage regime), the concentrations are below 20 ng/mL. Thus, mitoxantrone may apparently act via short-time immunosuppressive effects by the induction of cell lysis leading to both leukocyte reduction in the blood post infusion and inhibition of proliferation of all types of immune cells in vitro (53,54,58). In addition, a long-term immunological impact of mitoxantrone is considered to occur at lower and lowest concentrations by induction of programmed cell death in antigen-presenting cells (57). Consistent with this hypothesis, the clinical effects of mitoxantrone in MS have been suggested to last up to one year post-treatment (26).
Effects on the Cytokine Network

As early as 1980s, Fidler et al. (53) reported a decreased secretion of the pro-inflammatory cytokines interferon-\(\gamma\), TNF-\(\alpha\), and IL-2. In contrast, recent ex vivo analysis of the cytokine profile of immune cells obtained from patients before and during treatment with mitoxantrone revealed a decrease of IL-10 (an anti-inflammatory cytokine) expressing monocytes and of IL-2R-\(\beta\)1 expressing T-cells after six months of treatment (63).

CONCLUSIONS

For treatment of MS, immunosuppressive drugs including mitoxantrone have been used off-label for decades. Approval of immunomodulatory agents in the mid-1990s shifted the market towards interferon-\(\beta\) (see Chapter 14) and glatiramer acetate (see Chapter 15). However, worsening forms of RRMS and especially SPMS could not be treated satisfactorily with these new therapeutics. Thus, mitoxantrone that has immunosuppressive and also apparently immunomodulatory effects returned to the focus of interest which—based on its proven efficacy in phase III trials—has recently led to its approval.

The, thus far, positive experiences with mitoxantrone open further questions:

1. Can dose and frequency of administration be optimized?
2. Can the dose, due to the cardiotoxicity, be reduced after an induction phase without impairing the clinical effect?
3. Is there a rationale for a combination of mitoxantrone with immunomodulatory agents?
4. What is the optimal subsequent therapy after discontinuation with mitoxantrone?
5. What are the treatment options for clinical nonresponders to mitoxantrone?

In this circumstance, is there a rationale for the use of other immunosuppressants such as azathioprine or cyclophosphamide?

These and other questions are matter of intensive discussion. First preclinical and clinical studies including combination trials of mitoxantrone plus IFN\(\beta\), glatiramer acetate, or dexrazoxane have been initiated to address some of these aspects.

REFERENCES


INTRODUCTION

An ideal therapy for multiple sclerosis (MS), based on the widely held assumption that it is an organ-specific autoimmune disease, would selectively abolish the aberrant autoimmune response, while leaving the normal immune response against infections intact. Nonspecific immunosuppression is associated with substantial potential toxicity and generally marginal therapeutic effects. Recent research has enabled an understanding of many of the intricate mechanisms underlying the immune response in MS (see Chapter 4), and has stimulated approaches aimed at altering specific steps in the process. Some of the therapies based on these approaches have significant beneficial effects, advancing the battle to control systemically mediated inflammation in MS.

Targeted therapeutic strategies can be grouped into those that affect the initial events of antigen presentation to encephalitogenic T-cells, the activation of these cells, or their migration into the target central nervous system (CNS) tissue. We review some of the most recent therapies that have attempted to influence these steps through the use of monoclonal antibodies and T-cell based vaccination strategies and consider the results of the main trials with these agents.

MONOCLONAL ANTIBODIES

Monoclonal antibodies (mAbs) recognize and bind to a single structural motif on a specific antigen, generally with exquisite specificity. CD4+ T-cells, which recognize peptide fragments in the context of the major histocompatibility complex II molecule (MHC), orchestrate cellular and humoral immune reactions through the secretion of immunoregulatory cytokines and via cell to cell contact. Distinct types of helper CD4+ T-cells are identified based on their cytokine production. Th1-type cells secrete selected interleukins including IL-2, interferon gamma (IFNγ), and tumor necrosis factor alpha (TNFα), and are involved in cell-mediated immunity. Th2-type cells secrete IL-4, IL-5, IL-10, IL-13, and transforming growth factor beta (TGFβ)
and exert their primary function in humoral immune reactions and in modulating Th1 T-cell responses (1,2). In a number of experimental animal models of organ-specific autoimmunity, autoantigen reactive CD4+ Th1-type cells have been shown to be central for disease induction and progression (3,4). These cells are considered to play a pivotal role in a number of human autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease (IBD), and MS, and are therefore logical targets for intervention. Multiple mAbs with specificity for molecules expressed by Th1 cells including CD4, T-cell receptors (TCR), adhesion molecules, and costimulation receptors and others like cytokines involved in T-cell function have been studied. Several trials have evaluated the effects of some of these mAbs in patients with MS, in the hope of developing an immunologically specific, nontoxic form of therapy.

Murine mAbs that specifically deplete or interfere with the function of discrete T-cell subsets can prevent or delay the onset of experimental allergic encephalomyelitis (EAE) (5), and reverse signs of already established clinical disease (6). When administered to humans, murine mAb is a foreign protein that elicits a human anti-mouse antibody response (HAMA), which could block its therapeutic action (7). Chimeric mAbs are genetically engineered by combining a human constant region to the variable region of a murine antibody. Even when substantially molecularly engineered to remove all murine components except critical aminoacid sequences in the hypervariable regions of the mAb that provide the molecule its specificity of binding, these humanized mAbs may induce antibodies in humans, so called human antihuman antibody response (HAHA). Like HAMA, HAHA have the potential to limit or eliminate the effects of the administered mAb.

**Anti-adhesion Molecule Antibodies**

In inflammatory CNS disease, cell adhesion is an early step in lymphocyte and mononuclear cell migration across the blood–brain barrier. The massive infiltration of lymphocytes and monocytes that occurs into the early MS lesion is mediated by complex interactions, at first between low affinity adhesion molecules called selectins present on the surface of endothelial cells (P selectin and E selectin) and T-lymphocytes (L selectin). The expression of selectins on luminal surfaces of brain endothelial cells appears to be an inducible event triggered by tissue inflammation and orchestrated by cytokines, specifically IL-12 (8). These low affinity interactions are not sufficient for leukocyte arrest and transmigration into the CNS (8). Rather, subsequent steps depend on the activation of secondary adhesion molecules called integrins expressed on the lymphocyte surface, and interaction with their counterpart, the receptor ligands expressed on the endothelial cell surface (9). Integrins are transmembrane heterodimer receptors composed of noncovalently linked alpha and beta chains; they confer mechanical stability on interactions between cells and their environment and also act as cellular sensors and signaling molecules (10). The integrin–ligand interactions, most important in transmigration of lymphocytes across the blood–brain barriers are between two alpha-4 integrins, the first one very late antigen-4 (VLA-4) with vascular cell adhesion molecule-1 (VCAM-1) on the endothelial surface and the second one leukocyte function-associated integrin type-1 (LFA-1) with intercellular adhesion molecule-1 and -2 (ICAM-1, ICAM-2) on the endothelial surface (11,12). Once direct interactions have occurred between these molecule pairs changes in the endothelium’s cell junctions permit the transmigration of the leukocytes into the CNS where the inflammatory process continues. Although there are no human studies that confirm the above mechanisms, chronic MS lesions from
autopsy specimens do show high levels of expression of VLA-4 and VCAM-1 (13). Sera from MS patients also show increased levels of soluble endothelial ligands ICAM-1 when compared with controls; these levels coincide with the presence of gadolinium-enhancing lesions on brain magnetic resonance imaging (MRI) as well as clinical disease activity (14,15).

Targeting autoreactive lymphocyte trafficking with selective adhesion molecule (SAM) inhibitors intended to blockade receptor–ligand interactions is an elegant approach to reduce CNS inflammation in MS. The prototype integrin antagonists are mAbs. In animal models, mAbs with specificity for various adhesion molecules reduce cellular infiltration, inhibit the development of EAE, limit the progression of disease and even reverse existing symptoms by preventing inflammatory cells from crossing the blood–brain barrier. Some of these antibodies have entered human clinical trials for the treatment of autoimmune diseases such as IBD and MS (16,17).

**Natalizumab**

*Tysabri* (Biogen Idee Inc., Cambridge, MA, U.S.A., and Elan Pharmaceuticals, Inc., San Diego, CA, U.S.A.) is a humanized mAb created by grafting a murine antibody clone onto a human IgG4 framework at the complementary determining region (18). It is directed against alpha 4-integrins and binds to the alpha 4 subunit of alpha 4 beta 1-integrin (VLA-4) and alpha 4 beta 7-integrin expressed on leukocytes, blocking the interaction of these integrins with their vascular endothelial ligands, VCAM-1 expressed on brain endothelium and MAdCAM-1 expressed on vascular system of the gut; providing the rationale for its study in both MS and IBD. It has been proposed that natalizumab may have other properties based on observations on its therapeutic effect on Crohn’s disease, which is a neutrophil mediated disease, even though VLA-4 is not expressed on neutrophils. Alpha 4-integrins also interact with alternatively spliced domains of fibronectin, a molecule secreted in inflamed or injured endothelium, and found in the intact extracellular vascular matrix (18). These interactions are blocked in the presence of natalizumab. Alpha 4-integrins also interact with osteopontin, which has been shown to be a factor in the progression of EAE and perhaps relapsing–remitting MS (RRMS) (19). These additional ligands for alpha-4 integrins suggest there may be additional modulatory effects of SAM inhibition on the immune system.

**Clinical Trials.** A phase I, placebo-controlled, five-level dose-escalation study of single intravenous doses of natalizumab (0.3–3.0 mg/kg) evaluated 28 stable patients with RR or secondary progressive MS (SPMS). All doses were safe and well tolerated (20). In a subsequent phase II randomized, double-blind, placebo-controlled trial, 72 RR and SPMS patients were evaluated for acute effects of natalizumab on MRI lesion activity. Each subject received two intravenous infusions four weeks apart and was then followed for 24 weeks with serial MRI and clinical assessments. Over the first 12 weeks, those on active treatment exhibited significantly fewer new enhancing lesions, but no significant difference was seen between the natalizumab- and placebo-treated groups in the second 12 weeks of the study. The number of acute exacerbations was not different between groups in the first 12 weeks, but was higher in the treatment group in the second 12 weeks ($P = 0.005$), raising the suspicion for a rebound effect on natalizumab withdrawal (17).

In a second, larger phase II randomized, double-blind, placebo-control trial of 213 patients with actively relapsing MS, subjects received either 3 or 6 mg/kg
of natalizumab, or placebo every 28 days for six months. The primary outcome was the number of new enhancing lesions on monthly MRI; secondary outcomes included relapses and self reported well-being. Marked reductions occurred in the mean number of new lesions in both the natalizumab treated groups [9.6 per patient in the placebo group, 0.7 in the 3 mg/kg group (P < 0.001), 1.1 in the 6 mg/kg group (P < 0.001)]. Twenty-seven patients in the placebo group had relapses compared with 13 in the low dose group (P = 0.02) and 14 in the high dose group (P = 0.02). The placebo group reported a slight decrease in well-being, whereas the natalizumab groups reported some improvement. The natalizumab-treated patients showed a trend to a higher incidence of infections, especially pharyngitis. The treated patients showed elevated levels of lymphocytes, monocytes, and eosinophils but neutrophil levels did not change. The latter suggests that the antibody does not interfere with neutrophil functions necessary to combat bacterial and fungal infections. Binding antibodies against natalizumab (HAHA) developed at six months in seven patients (12%) in the low dose group and eight patients (11%) in the high dose group (21).

Another phase II study assessed the effect of a single dose of natalizumab administered soon after the onset of an MS relapse (clinical symptoms present for >24 hours but <96 hours, and expanded disability status scale (EDSS) score >3). This multicenter, double-blind, placebo-control study, randomized 180 patients in acute relapses to either 1 or 3 mg/kg of natalizumab or placebo and followed them for 14 weeks. No differences in the EDSS at weeks 1, 4, and 8 were found between the groups, and EDSS had improved an average of 1.6 points in all groups by week 8. Nevertheless, a significant decrease in enhanced lesion volume occurred in both treatment groups at weeks 1 and 3 compared with placebo (22).

In a study with similar design, a phase II trial of an anti-CD11/CD18 mAb in acute MS exacerbations (see also below) showed no apparent clinical effects on resolution of the relapse (23). While that mAb addressed a different integrin target, the results of both studies suggest that SAM blockade may have little apparent benefit when initiated after clinical symptoms appear, as the blocking of any additional leukocyte transmigration that occurs after the onset of the clinical attack may be “too late.”

A phase II randomized, double-blind, placebo-controlled trial involving 110 patients in 29 treatment sites evaluated the safety and tolerability of combination therapy with glatiramer acetate and natalizumab. The controlled phase lasted six months and was followed by a two year open label extension that was terminated pending a full safety review on recognition of progressive multifocal leuкоencephalopathy (PML) as a complication of natalizumab therapy (114–116). Table 1 summarizes human studies with natalizumab.

There were two completed phase III, 2-year, multicenter, randomized, double-blind, placebo-controlled trials. “AFFIRM” randomized 942 patients with RRMS and at least one relapse in the previous year to either 300 mg natalizumab or placebo (at a 2:1 ratio) given as an intravenous infusion every four weeks. The study had an unusual design with two different primary endpoints at different study epochs. The early primary outcome was the effect of natalizumab monotherapy on relapse rate, with secondary endpoints of new or newly enlarging T2 hyperintense lesions, the number of gadolinium enhancing lesions, and the proportion of relapse free patients at one year of therapy. The late primary outcome was the effect on accumulated disability as measured by change in EDSS from baseline at two years of therapy. In the other trial “SENTINEL”, the effect of 300 mg intravenous infusions of natalizumab
<table>
<thead>
<tr>
<th>Study</th>
<th>Duration</th>
<th>End point</th>
<th>Patients</th>
<th>Dose</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase Ia</td>
<td>4 wk</td>
<td>Single blind, five-level dose escalation, safety and pharmacokinetics</td>
<td>28 RR or RSPMS</td>
<td>0.03 to 3.0 mg/kg</td>
<td>All doses were safe and well tolerated</td>
</tr>
<tr>
<td>Phase IIb</td>
<td>24 wk</td>
<td>Acute effects on MRI lesion activity, relapse rate</td>
<td>72 RR or SPMS with relapses</td>
<td>3.0 mg/kg q mo × 2</td>
<td>Significant short-term reduction in Gd-enhancing lesions on treated patients. No effect on relapses</td>
</tr>
<tr>
<td>Phase IIIc</td>
<td>116 wk; then 2 yr open label</td>
<td>Effect on disease progression, relapse rate and MRI activity</td>
<td>942 RRMS with one relapse in the last year</td>
<td>300 mg IV q mo</td>
<td>One year data: 66% reduction in relapse rate and significant decrease in MRI activity. Two year data are pending</td>
</tr>
<tr>
<td>Phase IIIc</td>
<td>116 wk; then 2 yr open label</td>
<td>Effect on disease progression, relapse rate and MRI activity when combined with IFNβ-1a</td>
<td>1171 RRMS with one relapse a year on IFNβ-1a</td>
<td>300 mg IV q mo</td>
<td>One year data: 54% relapse rate reduction against IFNβ-1a alone and decrease in MRI activity. Two year data are pending</td>
</tr>
<tr>
<td>Phase IIIc</td>
<td>24 wk; then 2 yr open label</td>
<td>Efficacy, long-term effect on disease progression and relapse rate added to GA compared with GA alone</td>
<td>110 RRMS on GA within 12 mos</td>
<td>300 mg IV q mo</td>
<td>Pending</td>
</tr>
</tbody>
</table>

aPlacebo-controlled study.
bRandomized double-blind placebo-controlled trial.
cMulticenter randomized double-blind placebo-controlled trial.

Abbreviations: GA, glatiramer acetate; IFN, interferon; MRI, magnetic resonance imaging; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.
every four weeks was compared with placebo in 1200 RRMS patients with continuing relapses while on 30μg weekly intramuscular IFNβ-1a (Avonex®, Biogen Idec, Cambridge, MA, U.S.A.). Study design was otherwise the same as for the monotherapy trial (25).

The one-year data from the monotherapy study met the primary end point of clinical relapse rate reduction with a relative reduction of 66% compared with placebo. The annualized relapse rate for the placebo group (n = 315) was 0.74; for the treated group (n = 627) it was 0.25 (P < 0.0001). The percentage of placebo treated patients remaining relapse-free was 53%, and was 76% for the natalizumab treated group; statistically meeting the clinical secondary endpoint.

The MRI-based secondary endpoints showed the placebo group had developed a median of three new or newly enlarging T2 hyperintense lesions (mean 6.1), while the median was 0 for the natalizumab-treated group (mean 1.2). The percentages of patients with 0, 1, 2, and 3 or more lesions were 60%, 18%, 6%, and 16% for the natalizumab group, and 22%, 13%, 7%, and 58% for the placebo groups, respectively. The median for gadolinium enhancing lesions was 0 for both groups, with a percentage of patients with 0, 1, and 2 or more enhanced lesions at 96%, 3%, and 1% for the natalizumab subjects, and 68%, 13%, and 19% for placebo assigned subjects, respectively. The differences in these MRI-based endpoints were all highly significant.

The SENTINEL trial’s one-year analysis showed a 54% reduction in relapses when natalizumab was added to IFNβ-1a (n = 589), compared to treatment with IFNβ-1a alone (n = 582). The annualized relapse rate for IFNβ-1a plus placebo group was 0.78, and 0.36 for the IFNβ-1a plus natalizumab group. The percentage of relapse-free patients was 46% for the placebo-treated subjects and 67% for those treated with natalizumab. MRI-based endpoints for new or newly enlarging T2 hyperintense lesions showed a median of zero for the natalizumab-treated group and one for the placebo-treated cohort. The percentages of patients with zero, one, two, and three or more lesions were 67%, 26%, 4%, and 3% for the cohort receiving natalizumab and IFNβ-1a, and 40%, 29%, 10%, and 21% for those on placebo and IFNβ-1a, respectively. The median for gadolinium enhancing lesions was zero for both groups with the percentage of patients with zero, one, and two or more enhanced lesions at 96%, 3%, and 1% for those with added natalizumab, and 76%, 12%, and 12% for those on placebo and IFNβ-1a, respectively (25).

Natalizumab has a mean half life of 11 ± 4 days with a mean average serum concentration of 30μg/mL detectable over the dosing period. The current recommended dose is 300 mg by intravenous infusion every four weeks. The primary concern for therapy with natalizumab is the occurrence of allergic and hypersensitivity reactions (up to 7% of all subjects) including serious anaphylaxis/anaphylactoid reaction (0.8%). These usually occur within two hours of the start of intravenous infusions of the drug and appear to be more common in patients that develop antibodies to the drug. Patients experiencing these reactions are not to continue treatment with the drug. Other side effects are few and minor, although an increased rate of infections (urinary tract infections, lower respiratory tract, gastrointestinal system, and vaginal infections) may occur with this therapy. Preclinical studies in animals show that the drug undergoes transplacental transfer exerting a number of reversible hematopoietic effects on the fetus. The drug is likely to be excreted in human milk. It is a category C drug for use in pregnancy.

On November 23, 2004 the Food and Drug Administration (FDA) licensed natalizumab for the indication of treatment of patients with relapsing forms of MS to reduce the frequency of clinical exacerbations. The accelerated approval
was based on the results achieved after approximately one year of treatment (25). The identification of two patients in the extension phase of SENTINEL who developed PML (115,116) and a case of PML uncovered in a trial of natalizumab in Crohn’s disease (114) resulted in suspension of marketed drug in February 2006. A comprehensive safety analysis is under review by the FDA to determine if and under what conditions natalizumab might return to market in the United States.

Discussion. Although this novel treatment for MS appears to show superior efficacy compared with the existing therapies, it is important to consider other aspects involved with its use. Cross-study comparisons are always uncertain, however, the effect sizes of low dose IFNβ-1a when used for treatment of patients with clinically isolated syndrome were much larger than anticipated from studies in patients with well established relapsing disease. It is possible that the effect size for natalizumab, as monotherapy or add-on therapy, might reflect the subjects selected more than the true effect of the drug relative to other therapies. It is also possible that chronic inhibition of alpha 4-integrins might have undesirable events independent of its potential immunogenicity and HAHA induction for some exposed patients. Whether the emergence of PML will be limited to co-administration of natalizumab with beta interferons, remains a relatively infrequent and late complication of exposure to the drug, and whether it be the only opportunistic infection associated with the use of the drug in MS is uncertain. It is also of some concern that an embryonic deficiency in mice of either alpha 4-integrin or its counterpart VCAM-1 can be lethal before birth (10). This suggests that natalizumab should not be used during pregnancy. Cost and availability of this drug are other important practical issues. Nevertheless, considering the limited treatment options for MS the early experience with natalizumab is impressive and may if nothing else, set a higher bar for efficacy for a new generation of immunomodulators. A number of orally available small molecule alpha 4-integrin inhibitors are under development (26).

Other Anti-adhesion Molecule Antibodies

A phase I, uncontrolled, dose escalation study with humanized anti-CD11/CD18 mAb (HU23F2G) in 24 MS patients concluded that HU23F2G was tolerated at doses that achieved high degrees of leukocyte CD11/CD18 saturation and in vivo inhibition of leukocyte migration (27). A phase II, multicenter, randomized, double-blind, placebo-controlled trial of Hu23F2G was conducted in 169 patients with acute MS exacerbations. The efficacy of two different doses was compared with placebo as well as with intravenous high dose methylprednisolone (MP). Hu23F2G was ineffective in improving neurological status at a single dose of either 1 or 2 mg/kg. MP was associated with a greater decrease in brain MRI contrast enhancing areas (23). In a follow-up trial, three monthly doses of Hu23F2G started at the time of an acute attack also had little clinical effect on the attack, but enhancement frequency fell over the study with therapy (28). A phase II clinical trial assessing the efficacy of a mAb to CD18 (macrophage antigen-1) a leukocyte integrin similar to VLA-4 failed to demonstrate either clinical or MRI benefit in MS patients (29).

Other antibodies against adhesion molecules, including LFA-1 and MAC-1 were ineffective in inhibiting clinical symptoms and leukocyte infiltration in EAE (30,31). Anti-ICAM mAb showed modest benefit in a model of EAE, but the treatment resulted in intracerebral hemorrhages (32). Alternate adhesion molecules antagonists directed to the selectins, integrins, and receptor ligands continue to be investigated in animal models.
Anti-cytokine Antibodies

Cytokines play important signaling roles in cellular immune mechanisms. These soluble glycoproteins, nonimmunoglobulin in nature, act nonenzymatically to regulate immune cell function. As a potent mediator of inflammation, the cytopathic cytokine TNF appears to be important in the pathogenesis of both EAE and MS. A myriad of other cytokines undoubtedly influence the immune system and are potential targets of immune modulators with mAbs or soluble ligands.

TNF Antagonists

MAbs to TNF can prevent either active or passive transferred EAE in mice (33,34). Anti-TNF mAb effectively inhibits the development of EAE in SJL/J mice by interfering with the effector, rather than the induction phase of the disease (35). Murine-human chimeric anti-TNF mAb (CA2) was administered intravenously twice over a two week interval to two rapidly progressing MS patients. Clinical status, contrast-enhanced MRI, and peripheral blood and cerebrospinal fluid (CSF) immunologic status were monitored. Although clinically significant neurologic changes were not noted in either patient, the number of enhancing lesions increased as did CSF lymphocyte counts and the IgG index after each infusion in both subjects. This suggested that CA2 treatment might cause immune activation and an increase in disease activity (36).

Rat EAE was prevented by administration of P55–TNF-IgG fusion protein (TNFR-IgG). The hybrid molecule, while not an mAb, had similar properties and was felt to act by inhibiting an effector function of activated T-cells and possibly other inflammatory leukocytes (37). That neutralization of TNF by a recombinant TNF receptor P55 immunoglobulin fusion protein (lenercept) might reduce or halt MS progression was formally evaluated in a large phase II, randomized, multicenter, placebo-controlled study of 168 patients, most with RRMS. Patients received infusions of 10, 50, or 100 mg of lenercept or placebo every four weeks for up to 48 weeks. The number of treated patients experiencing exacerbations was increased compared with the placebo group ($P = 0.007$), and their exacerbations occurred earlier ($P = 0.006$). Perhaps paradoxically, there was no significant difference between the groups on any MRI measure. Antifusion protein antibodies were present in a substantial number of treated patients (38). The recombinant TNF receptor P55 immunoglobulin fusion protein had repeatedly shown potent preventive and therapeutic effects in various EAE protocols. However, those results were not predictive of the drug effect actually observed in MS.

IL-10 Antagonists

IL-10 is a Th2 immunomodulatory cytokine with known downregulatory effects on Th1 responses and macrophages. In murine EAE, the administration of anti-IL-10 mAb had no effect when given early postsensitization and it caused marked worsening when given immediately before clinical onset of the disease (39).

IFNγ Antagonists

IFNγ is regarded as a proinflammatory cytokine and has been shown to induce relapse in MS patients (40). Hence an mAb to this cytokine might be expected to lessen the severity of EAE. Paradoxically, four independent groups have shown that such a treatment exacerbates both active and passive transfer EAE (41–44). Because of the intricate balance of cytokines in maintaining immune regulation, the partial
redundancy in their effects, fluctuations in the balance during beneficial or autodestructive immune responses, and the different effects that reduction of the effects of a cytokine might have on immune damage, cytokine specific immune therapy may be less predictable than originally anticipated. Nevertheless, attempts to manipulate this system continue.

**IL-2 Antagonists**

Anti-IL-2 mAbs have a beneficial effect on passive EAE, but not on active disease (45, 46). Daclizumab (Zenapax® Roche Labs, Nutley, NJ, U.S.A.) is a humanized mAb specific for the IL-2 receptor alpha chain (IL-2Ra) that inhibits IL-2 mediated activation of lymphocytes. It is FDA approved for the prevention of renal transplant rejection. A recent phase II, open label, baseline-to-treatment trial, evaluated the safety profile and efficacy of adding daclizumab to patients with incomplete response to therapy with IFNβ-1b. Failure to respond to IFN was defined as at least one exacerbation or progression of EDSS by at least one point during the preceding 18 months of therapy. Eleven patients with RR or SPMS were enrolled and followed by monthly clinical and MRI examinations while they continued on IFNβ-1b monotherapy for four additional months. To be eligible to initiate therapy in a given subject, at least 0.67/month new contrast enhancing lesion (CEL) during this baseline period was stipulated. Daclizumab was administered intravenously at 1 mg/kg/dose given two weeks apart for the first two doses then every four weeks thereafter for a total of seven infusions. Overall the drug was well tolerated. Efficacy was measured by MRI inflammatory activity. The results included a 78% decrease in new CEL and 70% decrease in total CEL as compared to baseline; this relative decrease occurred gradually over two months. The results of secondary outcomes including T2 lesion volume, black hole volume, EDSS, and timed 25 foot walk were not significant, whereas the volume of CEL (73% reduction), exacerbation rate (81% reduction), Scripps NRS (9%) and 9-hole peg test (5%) improved significantly (47).

In another open label study, 19 ambulatory patients with clinically active RR and SPMS were treated for 5 to 25 months (average 13.6 months); 17 of the patients were felt to be nonresponders to other immunotherapies, two continued IFNβ-1b therapy for the first six months and one continued monthly methylprednisolone infusions. All subjects received 1 mg/kg daclizumab intravenously followed by a second dose after 14 days and then every 28 days adjusted based on clinical response to between 0.8 and 1.9 mg/kg per infusion. Daclizumab was generally well tolerated. Sustained clinical improvement (10 patients) or stabilization (nine patients) was observed. MRI activity was also felt to improve following therapy (48). These intriguing open label studies require confirmation in rigorously designed, controlled and blinded trials.

**Miscellaneous**

A phase II trial to evaluate safety and effectiveness of human anti-IL-12 (ABT-874) is ongoing, as are trials on various down-modulatory cytokine inhibitors for IL-1, IL-4, IL-10, and IL-13 (2). Other potential targets for mAb antagonist therapy include the chemokine receptors of which CCR5, CXCR3, CCR1, and CCR2 appear to be most relevant due to their role in enhancing integrin binding affinity to the vascular endothelium (49). These are all currently being investigated in phase II trials.
Anti-CD4 and Related Antibodies

CD4 is a cell surface antigen found almost exclusively on the helper subset of T-lymphocytes. Since the pathogenesis of EAE and presumably MS involve CD4⁺ Th1 T-cells that control many aspects of immune function, CD4 is a logical target for intervention. Anti-CD4 antibodies are effective in reversing various spontaneous and induced animal models of autoimmune diseases, including advanced clinical stages in nonhuman primates (50). Very early work with murine anti-CD4 mAb showed that it prevented the development of EAE (6). Treatment with anti-CD4 mAb reverses EAE even when given to paralyzed animals. Anti-CD4 mAb selectively depletes CD4 bearing T-cells from lymph nodes and spleen in mice (6), but did not appreciably deplete CD4 T-cells in the rat EAE model (5). However, treatment with both CD4⁺ cell depleting, or CD4⁺ blocking/nondepleting mAb inhibited disease progression in mice with chronic relapsing EAE (51). In Lewis rats, anti-CD4 mAb does not ablate the encephalitogenic CD4⁺ cells or prevent the development of resistance to EAE, but it may inhibit EAE by preventing the function of already activated effector cells (52). EAE in primates is often quite severe when compared with that seen in the rodent models. Treatment of Rhesus monkeys with OKT4⁺4A (anti-CD4 mAb) can reverse clinical signs of EAE (50). Outbred long tailed Macaques on anti-CD4 mAb treatment showed prolonged survival and in some cases complete reversal of clinical EAE (53). However, of caution, mice with chronic CNS toxoplasmosis develop fatal disease when treated with anti-CD4 mAb. This therapy, while targeted to a very specific T-cell sub-population, induces wide-spread immunodeficiency. Pretreatment with a nondepleting anti-CD4 mAb (H129.19) that produces long-lasting receptor saturation, fully protected PL/J mice from EAE (54). These results further illustrate the varied mechanisms through which mAbs directed at the same molecule may exert their effects.

Phase I trials were conducted in chronic progressive MS patients with anti-T12, anti-T4, and anti-T11 mAbs. Anti-T11 mAb decreased T-cell activation by phytohemagglutinin and anti-T4 mAb infusions abolished pokeweed mitogen induced immunoglobulin synthesis without lyses of the CD4⁺ T-cell subpopulation (7). In early trials in chronic progressive MS patients, five daily infusions (0.2 mg/kg/day) of either murine anti-CD2 (an antigen more widely expressed on T-cells) or anti-CD4 mAb were reportedly tolerated well (55). Eighteen hours after the first anti-CD4 mAb infusion, there was approximately 50% decrease in circulating CD4⁺ lymphocytes accompanied by a twofold increase in the percentage of circulating CD8⁺ cells and several in vitro measures of the immune response were suppressed. Most of these subjects developed HAMA primarily of the IgG isotype (7).

A phase I open label trial with murine anti-T CD4/BF5 mAb was done in 35 patients with active MS (18 progressive and 17 RR). Therapy induced a marked CD4⁺ lymphocyte depletion. Only minor general side effects were noted in 22 patients and only upon the first mAb infusion. These may be related to the release of cytokines from T-cells stimulated by the mAbs. One year later functional disability was stabilized in only 6 of the 35 patients, and after two years in only 2 of 21 patients. No changes in the lesions were noted on MRI scans performed after treatment (56).

Single and repeated infusions of a chimeric murine/human anti-CD4 mAb (CM-T412) resulted in a profound selective depletion of CD4⁺ cells in phase I studies in MS patients (57,58). This led to a randomized, double-blind, placebo-controlled, MRI-monitored phase II trial in 71 patients with active RR and SPMS.
However, there was no significant effect on the primary measure of efficacy and the number of enhanced lesions on monthly MRI over nine months. There was a 41% decrease in the number of clinical relapses (secondary efficacy parameter) after nine months ($P=0.02$) (59). Failure of the mAb to delete primed IFN$\gamma$ producing T-cells correlated with therapeutic failure (60).

**Alemtuzumab**

CD52 is a surface antigen found on T-cells and macrophages. In a pilot study, seven MS patients treated with alemtuzumab (Campath-IH®, Berlex, Montville NJ, U.S.A.), a humanized anti-CD-52 mAb, had a substantial reduction in disease activity as measured by enhancing lesions on MRI (61). Five-day pulse treatment of 27 MS patients with alemtuzumab in an open label clinical trial depleted 95% of circulating lymphocytes. CD4 and CD8 counts were 30% to 40% of pretreatment values 18 months later. One-third of these patients developed antibodies against the thyrotropin receptor and carbimazole-responsive autoimmune hyperthyroidism. Altogether 12 out of 37 alemtuzumab-treated MS patients developed Graves disease, while this complication was not reported among 600 alemtuzumab-treated patients for various other disorders. This suggests that patients with MS may be uniquely susceptible to this complication (62). The earlier report concluded that a single pulse of alemtuzumab suppressed MRI markers of MS disease activity for at least six months. An extended follow-up of 27 additional patients showed MRI markers of disease activity were significantly suppressed for at least 18 months in all patients, yet half experienced progressive disability. The investigators suggested that this was probably due to axonal degeneration conditioned by high pretreatment disease activity. Alemtuzumab causes the immune response to change from the Th1 phenotype, suppressing MS disease activity, but permitting the generation of antibody-mediated thyroid autoimmunity (63). In a crossover treatment trial in 25 SPMS patients, treatment was associated with a reduction in the number and volume of enhancing lesions ($P < 0.01$), but a decrease in brain volume was seen in 13 patients during the 18 months post-treatment period (64).

In a recent publication by the Cambridge group, who report their complete experience of the use of Campath-1H since 1991, the investigators summarize that clinical and radiological data from 58 patients with SPMS suggest that just one or two pulses of the drug significantly suppresses cerebral inflammation for at least six years. The 58 patients experienced only 11 episodes during 275 patient-years of follow up during both the RR (32 years) and the SP (243 years) phases of the disease. However, there was evidence of cerebral atrophy at a volume loss of $1.37 \pm 1.28$ mL per year. They concluded that once the cascade of events leading to tissue injury is established, effective suppression of inflammation does not limit brain atrophy or protect from clinical progression, and that any opportunity to alter these may be only early in the disease course (65). The drug is now under investigation in more rigorously controlled and randomized studies of relapsing forms of MS.

**Anti–T-Cell Receptor Antibodies**

Immunoglobulins and their close relatives, the antigen-specific TCR, are recognition proteins that express structures that readily serve as self-immunogens. Healthy humans can produce antibodies against variable region defined recognition
structures termed idiotypes, as well as against constant region structures, and the level of these can increase markedly in autoimmune disease. Most recent analyses employing synthetic peptide technologies and construction of recombinant TCR document that autoantibodies directed against both variable and constant region markers of the TCRαβ occur in healthy individuals. Two of the major autoimmunogeneic regions of the TCRαβ are “constitutive” markers in that all individuals tested produce antibodies against these regions. Alterations in levels of antibody, usage of IgM or IgG isotypes, and specificity for particular peptide-defined regions vary with natural physiological processes such as aging and pregnancy, with artificial allografting, with retroviral infection, and with the inception and progression of autoimmune diseases. The most frequently observed autoantibodies are against TCRβ CDR1 and Fr3 markers. It is hypothesized that these are normally involved in immunoregulation.

The natural tendency in T-cell mediated autoimmune conditions to develop focused antigen-specific responses that overutilize certain TCR V region segments prompted the induction of anti-TCR specific T-cells and antibodies that can inhibit the pathogenic T-cells and promote recovery from disease. In some strains such as the Lewis rat and the PL/J mouse, the encephalitogenic MBP-specific T-cells over-express a particular V region gene (BV8S2) of the TCR (66,67). Administration of a combination of anti-BV8S2 and anti-BV13 mAbs results in a long-term elimination of T-cells involved in the response to MBP in B10.PL mice. When given before MBP immunization, anti-TCR antibody treatment leads to nearly complete protection against EAE, and a dramatic reversal of paralysis in diseased mice (68). SJL/J mice with relapsing EAE induced by a PLP 139–151-specific T-cell line expressing 88% BV2 when treated with anti-BV2 mAb at the time of cell transfer or at clinical disease onset exhibit markedly reduced clinical and histological disease severity (69). R73 is an mAb specific for rat TCRβ that administered at low dose protects rats from EAE. When treatment was started shortly before the onset of clinical signs, R73 completely suppressed the induction of EAE, and when started on the day of onset of clinical signs, it hastened recovery (69).

Specific TCR mAbs have not been directly administered to MS patients, but the induction of polyvalent anti-TCR antibodies by TCR peptides might contribute to the responses seen in MS patients treated in several peptide pilot studies (discussed further below).

Antibodies to Costimulation Receptors

Antigen bound to MHC alone is not sufficient to activate T-cells. The TCR must not only contact the antigen in the MHC antigen binding groove, it also requires a concurrent second signal or costimulation from antigen presenting cells (APC). When T-cells are activated, they express cell surface molecules that are not present on naïve cells. In autoimmune disease, the autoreactive cells will express activation antigens whereas normal cells do not. Autoreactive cells would be selectively eliminated by cytotoxic mAb against the activation antigen given at this time.

Several studies have shown that direct interference with the interaction of B7 (a macrophage membrane bound cell surface antigen) and CD28 (a T-cell surface antigen) disrupts a costimulatory pathway and leads to antigen-specific unresponsiveness. Interference with B7/CD28 is an effective means of preventing induction of relapsing EAE (70,71) and of treating ongoing disease (72,73). A phase I trial was completed on the safety of CTLA4-lg, a recombinant protein of
cytolytic-T-lymphocyte associated antigen-4 fused to the heavy chain constant region of the human immunoglobulin of IgG4 isotype. The gene sequence encoding the immunoglobulin portion was altered to remove the functional properties of Fc receptor binding and complement fixation. CTLA4-lg blocks CD28-B7 costimulation. Unfortunately, a phase II trial evaluating efficacy was prematurely terminated for as yet undisclosed reasons (74). CD40 although originally identified as a constitutive B-cell antigen is expressed by many cells, including dendritic cells, macrophages, and astrocytes. CD154 (CD40L), the ligand for CD40, is transiently expressed primarily by activated CD4+ T-cells. Recently CD154 was identified on a subpopulation of activated B-cells. CD40 ligation leads to upregulation of costimulatory molecules B7-1 and B7-2 on the APC, enhancing their ability to activate naïve T-cells. CD40–CD154 interactions are crucial for B-cell activation and differentiation and for production of IL-12 by APC, which biases CD4 T-cell responses toward Th1. CD40–CD154 interactions may be involved in directing CNS migration of encephalitogenic cells and/or in their ability to activate CNS macrophages/microglia. When anti-CD154 mAb was administered to SJL mice at either the peak of acute disease or during remission, clinical disease progression, and CNS inflammation was effectively blocked. The proportion of anti-CD 154 mAb treated mice with relapses (37%) was significantly reduced compared with that for control mice (81%). In vitro T-cell proliferation assays showed that anti-CD154 treated animals with ongoing relapsing EAE had inhibited Th1 responsiveness and epitope spreading (75). An open label trial to evaluate the safety of CD40 (CD154) in humans is currently ongoing.

Anti–B-Cell Antibodies

In primary progressive MS (PPMS) a postulated mechanism of CNS damage may be mostly a prolonged antibody rather than T-cell mediated immune attack, based on the circulating antigen specific antibody levels persistently elevated in PPMS patients as opposed to the marked fluctuations that occur in the levels of circulating antigen-specific T-cells. Recent evidence indicates that an increased CSF B-cell to monocyte ratio correlates with disease progression in MS (76), and that the presence of intrathecal IgM synthesis in RRMS predicts secondary progression (77). These findings raise the possibility that disease progression is related to antibody mediated CNS damage. Patients with PPMS have increased serum antiganglioside antibody levels compared with RRMS patients and controls (78). The patients with SPMS have intermediate levels (79). Furthermore, antibodies to light the neurofilament subunit, an axonal cytoskeletal protein, are also increased in patients with PP or SPMS compared with RRMS. Future better definition of these antibodies might lead to advances in the diagnosis of PPMS.

In a very small, open-label study, the administration of 375 mg/M² of rituximab to four subjects with neuromyelitis optica and four rapidly progressing RRMS subjects resulted in a dramatic fall in B-cells in the peripheral blood, and an apparent clinical response for most patients (80). On the basis of the above study, it has been proposed that rituximab (Rituxan®, Genentech, San Francisco, California, U.S.A.), an mAb against B-cells originally approved for the treatment of B-cell non-Hodgkin lymphoma, may slow the progression of MS by depleting B-cells; this may be effective by preventing antigen presentation to T-cells in the CNS and preventing autoantibody production. A phase III trial that projects to enroll 435 PPMS subjects is currently recruiting patients, and a trial in RRMS has been proposed.
T-CELL VACCINES

Substantial evidence indicates that MS may have an autoimmune component, mediated by autoreactive T-lymphocytes specific for myelin antigens. The putative T-cell autoantigens remain uncertain, but MBP and myelin oligodendrocytic glycoprotein (MOG) are two major candidate autoantigens. Clonally expanded MBP-specific T-cells persist for several years in the blood of MS patients and activated MBP specific T-cells migrate and accumulate in the CNS, where they have been identified in brain lesions of MS patients (81–84). It is not yet clear how these T-cells are initially activated, but several studies suggest that viral antigens mimicking myelin epitopes may be involved. Moreover, there is evidence that regulatory mechanisms that control autoreactive T-cells in healthy subjects are potentially defective in MS patients. In addition to myelin reactive T-cells, B-cells producing myelin-specific antibodies and γδ T-cells may also play important roles in the autoimmune cascade (85). As MBP-reactive T-cells may be key in the initiation and perpetuation of the CNS inflammation in MS, specific immune therapies have been proposed to deplete them in attempts to improve the clinical course of the disease (86).

T-Cell Receptors

The TCR is a complex transmembrane molecular subunit of the T-cell that distinguishes it from other T-cells. The TCR, like the immunoglobulins, has both constant and variable complementary regions and is selected under the pressure of antigenic stimulation. The progeny of a given T-cell clone has a unique TCR and limited antigen specificity. While once felt to be entirely specific for a given epitope, some limited cross reactivity is now established in a manner analogous to the limited degeneracy of some mAbs. The TCR approach in MS and other putative autoimmune diseases assumes that (i) the subpopulations of the putative autoaggressive effector T-cells must utilize only a limited number of TCR genes, (ii) the T-cell vaccine must provoke an immune response that recognizes the naturally occurring TCR peptide fragment present in the context of MHC II on the surface of disease causing T-cells, and (iii) the resulting immune response must somehow inhibit or downregulate the activities of disease-causing T-cells in a manner sufficient to provide a clinical benefit without toxicity or undesirable effects. An understanding of the immune response against the TCR hypervariable region fragment is a prerequisite for successful TCR vaccination therapy of MS and other antigen-specific autoimmune diseases (87).

T-Cell Receptor Studies

The subpopulation of T-lymphocytes responsible for EAE in the Lewis rat utilizes the TCR BV8S2 region gene (88–91). Treatment with a vaccine consisting of a peptide fragment of VB8S2 reduced the level of CNS inflammation and the severity of paralytic disease in the rat EAE model (92,93). Depletion of these cells by treatment with a VB8S2 specific mAb either before or after immunization with MBP also significantly reduced disease severity (94). These and similar studies suggest that immunization to deplete a population of T-cells that contain putative encephalitogenic autoreactive T-cells can control EAE, whether mediated by anti-TCR antibodies, or by regulating or cytotoxic T-cells. TCR peptide vaccine approaches have differed in ways that may vary their effects based on the specificity of action of each in the immune cascade (95).
The first study to assess the safety and immunogenicity of TCR peptide (96) evaluated chronic progressive MS patients treated with CDR2 region peptide of TCR BV5S2 or BV6S1 (this sequence is expressed in MS plaques and on MBP-specific T-cells). No toxicity was observed and treatment did not cause broad immunosuppression. Some of the treated subjects developed delayed-type skin reactivity and TCR peptide specific-antibodies.

Subsequently, a double-blind pilot trial with TCR BV5S2 peptide vaccine was conducted in patients with progressive MS. Vaccine responders had a reduced MBP response and remained clinically stable without adverse effects during one year of therapy, whereas the nonresponders had an increased MBP response and progressed clinically. Peptide-specific Th2 cells directly inhibited MBP-specific Th1 cells in vitro through IL-10 release, implicating bystander suppression (97). An as yet not formally reported, multicenter, placebo-controlled trial of these TCR peptides in 106 MS subjects apparently developed similar results (87). Approximately half of all subjects immunized with native peptide or site substituted versions of the TCR BV5S2 peptide vaccine develop measurable responses.

A more recent phase II trial evaluated antibody (ATM-027) mediated suppression of BV5S2/BV5S3þ T-cells with MRI monitoring on 47 MS patients versus placebo (98). Consistent T-cell suppression was found for the treated patients. However, the effect size on MRI was marginal at only 10%.

The experience of the Portland group has prompted them to seek more effective vaccination approaches including exploring the use of adjuvants. A T-cell receptor peptide vaccine (Neurovax™, Immune Response Corporation, Carlsbad, California, U.S.A.) composed of a combination of CDR2 TCR peptides from three families (BV5S2, BV6S5, and BV13S1) in incomplete Freund’s adjuvant (IFA) was evaluated in a phase I/II trial. The purpose was to compare the immunogenicity of this tripeptide vaccine together with an adjuvant with the one observed in the single TCR peptide vaccine reported earlier. In this study, 37 patients with confirmed MS were randomized to the three peptides with saline given intradermally (n = 15), the vaccine with adjuvant given intramuscularly (n = 16), or intramuscular adjuvant alone (n = 6). All subjects received monthly injections for 24 weeks. The primary outcome measure was the fraction of subjects immunologically responding to peptides measured by having >2 postimmunization reactive T-cell frequencies significantly higher than the preinjection frequency and >1 postimmunization frequency >2 cells/10⁶ peripheral blood mononuclear cells by limiting dilution assay. The study was discontinued after 24 subjects completed the protocol when an interim analysis disclosed that the primary outcome had been met. Using an intent-to-treat analysis, the fraction of subjects who were TCR responders was significantly greater in the TCR tripeptide with IFA group (15/16; 94%), compared with for the tripeptide in saline (1/15; 7%), or IFA alone (0/6; P < 0.001). MRI was done at weeks 16, 20, and 24. There was a trend favoring decreased MRI activity among TCR peptide responders. Only site reactions were reported as adverse events (99). Assessment of clinical effectiveness of this vaccine may be warranted.

A phase I trial of a TCR BV6S5 CDR2 region peptide vaccine was conducted in 10 MS patients with biased over representation of VB6 mRNA among T-cells isolated from their CSF (100). These patients were monitored for adverse events, immunogenicity of the peptide, and changes in their CSF T-cell populations. The peptide was found to be immunogenic in some patients, although none of the immunized patients produced detectable antipeptide antibodies. Five patients treated with 300 µg of vaccine displayed a slight decrease in CSF cellularity and a lack of growth.
in CSF cells in cytokine supplemented expansion cultures. This implied an absence of a subset of activated CD4+ T-cells and a reduction of BV6 mRNA levels among T-cells in these cultures. In the five patients who received the 100 µg vaccine dose, CSF cellularity was the same or slightly increased over prevaccination levels. CSF cells from one patient failed to grow in expansion cultures and cultured cells from two low dose patients underwent a change from an oligoclonal BV6 pattern to one that was more polyclonal. This clonal prevalence and over representation of BV6 raised the possibility that immunization with a BV6 peptide vaccine might produce a regulatory immune response. In a related study, 8 of 10 MS subjects immunized with 300 µg of a BV6S2/BV6S5 peptide vaccine developed evidence of cellular reactivity to the peptide (101).

A widely active vaccine for MS might involve a limited set of slightly modified CDR2 peptides from BV genes involved in T-cell recognition of MBP (87).

### T-Cell–Based Vaccines

The concept of T-cell vaccination in MS is similar to that of attenuated vaccines used against microbial agents in infectious diseases. T-cell vaccination is a procedure whereby MS patients are immunized with attenuated autologous MBP reactive T-cells, which induces an immune response to the vaccine cells and consequently a depletion of MBP reactive T-cells (102).

Originally six MS patients were inoculated with autologous attenuated MBP-specific T-cell clones three times at two-month intervals. No toxicity was observed, and after the final inoculation the precursor frequency of the MBP-specific T-cells dropped to undetectable levels in all patients. Limited antiergotypic and pronounced anticonotypic T-cell responses were seen. This clinical trial showed that antigen-specific T-cell vaccination was feasible in humans (103). A subsequent study demonstrated that MBP-reactive T-cells remained undetectable in the circulation of six of nine T-cell vaccine recipients for one to three years after vaccination. However, they reappeared in some individuals coinciding with clinical exacerbations (104). In another pilot trial, eight MS patients received vaccination with irradiated T-cells reactive to MBP. Compared to their two-year prior exacerbation rates, attack frequency decreased in five vaccinated patients with relapsing–remitting disease from 16 to 3, and from 12 to 10 in their matched, but not randomized controls (105). MRI showed a mean 8% increase in brain lesion load in vaccinated patients compared with 39.5% increase in the control cases.

An extended phase I, uncontrolled trial was done on 49 MS patients in Belgium and Houston to study the safety, immune responses and clinical effects of T-cell vaccination (102,106–108). Substantial long-term in vitro proliferative responses were observed in all treated patients. Autoreactive CD8+ and CD4+ αβ T-cells, and to a lesser extent γδ T-cells and NK cells were observed to in vitro stimulation with vaccine cells. Thus, immunization with attenuated autoreactive T-cells induced a complex cellular response specifically targeted at vaccine cells. Longitudinal clinical evaluation suggested a possible reduction of rate of clinical exacerbation, disability score, and MRI brain lesions in vaccinated patients (107,108).

Efforts to dissect the induction mechanisms of the anti-idiotypic T-cell response after T-cell vaccination concluded that the response is associated with TCR peptides corresponding to a common CDR3 (and to a lesser extent CDR2) sequence motif in MBP reactive T-cells (109). B-cells producing anti-idiotypic antibodies were also isolated from vaccinated patients that reacted with and inhibited
proliferation of the original immunizing T-cell clones. Importantly they were also found to react preferentially with CDR3 sequences of the immunizing cells (110).

A more recent open label study of 28 RR and 26 SPMS subjects compared relapse rate, EDSS progression, and MRI activity over 24 months of quarterly treatments with irradiated autologous T-cells with the patient's clinical course and MRI activity for the 24 months before vaccine therapy was initiated. No differences were found in the precursor frequency of circulating MBP-reactive T-cells detected at baseline between the RR and SPMS groups and these were similar to previous studies done by this group. More than 90% of the patients developed T-cell responses to the immunizing cells after the second and third vaccination. Complete depletion of MBP-reactive T-cells was reported in 92% of patients with significant declines in the remainder two to three months after the last vaccination (111).

Clinical results included minimal improvement in the RRMS patients (EDSS change from 3.2 to 3.1) and minimal progression in the SPMS patients. Approximately 20% of patients in both groups progressed, and this appeared accelerated 18 months after beginning vaccination, correlating with findings of MBP-reactive T-cells in 10% to 20% of the patients measured at around the same time. Importantly, these cells were from a different clonal population suggesting clonal shift or epitope spread that could also explain the possible decrease in vaccine effectiveness. The annual exacerbation rate decreased by 40% in patients with RRMS compared with baseline. Although the relapse rate decreased by 50% in SPMS patients these results are difficult to interpret since only six had a relapse in the two years prior to study entry. No significant difference was found between the relapse rate in the first and the second year of the study. MRI done at baseline, 12 and 24 months for 34 subjects showed a 1.2% group reduction in activity in the mean MRI lesion score in the first year and a 3.3% increase in the second year (111).

A pilot study of vaccination with autologous CSF-derived activated T-cells showed good tolerability. On the basis of this, a double-blind, placebo-controlled trial to study the effects of this type of T-cell vaccine is ongoing on 60 MS patients (112).

Another NIH funded study evaluated T-cell vaccination against whole bovine myelin. This was a double-blind, placebo-controlled trial of 80 SPMS patients. The purpose of the study was to control lesion development and disease progression as well as to determine the impact of vaccine on immune function. Outcome measures included cerebral MRI, EDSS, and immune parameters. Patients were given a subcutaneous injection of $40 \times 10^6$ lymphocytes 11 times over 24 months and were to be observed for a total of three years. The study was terminated prematurely due to lack of apparent clinical effectiveness over placebo (113).

CONCLUSION

The TCR-peptide immunotherapy and T-cell vaccination are primarily designed to target the TCR of MBP-reactive T-cells. The TCR V gene repertoire of MBP auto-reactive T-cells varies considerably among patients with MS. No common TCR V gene pattern has emerged for the disease association. Thus, the heterogeneous expression of TCR V gene products among a general MS population complicates attempts to develop an immunotherapy directed at a “common” variable regions of the TCR. A treatment agent designed to target certain TCR V gene products may be useful in one patient, but not in others, hampering its clinical usefulness. Also
evidence of clonal shift and/or epitope spreading of the postvaccination MBP-reactive T-cell lines complicate the long-term usefulness of T-cell vaccines.

Attenuated T-cell vaccines induce complex cellular and humoral responses specifically targeted at the immunizing clones, but do not affect MBP-reactive clones that are not part of the immunization. Despite promising results of the pilot trials, rigorous proof of efficacy both short and long term are still lacking. Recent studies showing specific details on the postvaccination immune response mechanisms provide grounds for further investigations, not only on the treatment efficacy of T-cell and TCR peptide vaccination but also on the regulatory mechanisms of autoimmune T-cells and the possible reasons for their dysfunction.

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Immune Therapy for Multiple Sclerosis: Altered Peptide Ligands and Statins

Fu-Dong Shi, Denise I. Campagnolo, and Timothy L. Vollmer
Barrow Neurological Institute, St. Joseph’s Hospital and Medical Center, Phoenix, Arizona, U.S.A.

INTRODUCTION

Immune therapies in multiple sclerosis (MS) can be generally classified as “antigen-specific” and “antigen nonspecific.” Antigen-specific therapies refer to tolerance induction via administration of native myelin antigens or altered peptide ligand (APL). Analogs of immunogenic peptides containing substitutions at T-cell receptor (TCR) contact residues are defined as APL. There is some evidence, which suggests that APL can induce bystander-suppression. This mechanism explains why a given myelin antigen or an APL with limited immunogenic determinants capable of suppressing MS patients whose multiple myelin antigens are targeted by T-cells. Antigen nonspecific therapies include several Food and Drug Administration (FDA) approved therapies (e.g., interferon IFNs, Natalizumab, or Tysabri) or therapies being vigorously tested (e.g., statins). These reagents aim to correct the immune aberrance of MS patients in a nonantigen specific manner. This chapter discusses the emerging evidence that APLs and statins may have beneficial effects in MS patients. Current status of clinical trials, using APL and statins, is also discussed.

IS THE USE OF APLs STILL A VIABLE APPROACH FOR TREATMENT OF MS?

Concept of APLs

Analogs of immunogenic peptides containing substitutions at TCR contact residues are defined as APL (Fig. 1) (1,2). APL bind to the major histocompatibility complex (MHC) with an affinity comparable to that of the native peptide but are not recognized “appropriately” by autoreactive T helper (Th) cells. The inappropriate recognition of an APL by autoreactive T-cells could lead to anergy, apoptosis, or alteration in the cytokines released from these T-cells (e.g., bystander suppression) (Fig. 1). In a broader sense, many therapeutic strategies involving agents that compete with the process of recognition of (neuro)-antigens by T-cells (e.g., copolymer-1,
peptides, oligomers) fall into this category. The fact that many autoimmune diseases are preferentially associated with a specific cluster of MHC class II molecules (e.g., over 50% of MS patients are HLA-DR2 positive) makes this approach feasible. APL have been used to manipulate antigen-specific T-cell responses in autoimmune diseases, including experimental autoimmune encephalomyelitis (EAE) and MS (2, 3). In addition to their usefulness in controlling autoimmune disorders, APL may prove helpful for the treatment of many diseases such as cancer, immunodeficiencies, and asthma. That is, learning to downregulate Th1 responses with APL could be beneficial whenever a Th2 bias is desirable.

**Figure 1** Putative biological effects of altered peptide ligand. Antigen-presenting cells can deliver cognate peptide antigen to trigger T-cell activation, proliferation, differentiation, and effector function. The cardinal step for initiation of autoimmune disease might be the commitment of naïve T-cells (Th0) to differentiate into proinflammatory (Th1) or anti-inflammatory (Th2) cells. Such processes are influenced by a unique type of antigen-presenting cells, the expression of costimulatory molecules and cytokines such as IFN-γ, IL-12, and IL-4. Altered peptide ligand are analogs of immunogenic peptides containing substitutions at T-cell receptor contact residues. In a broader sense, many therapeutic strategies involving agents that compete with the process of recognition of neuroantigens by T-cells (e.g., copolymers, peptides, oligomers) fall into this category. Depending on the substitution(s) at the T-cell receptor contact residues of cognate antigen, an altered peptide ligand can act as an antagonist; consequently, T-cells fail to proliferate and become anergic (anergy). Altered peptide ligand can also act as a partial agonist, eliciting some but not all functions. This may result in reduction of proliferation, polarization toward a subset of T-cells that secrete specific cytokines (immune deviation), or induction of altered peptide ligand-specific regulatory Th2 cells that cross-react with cognate self antigen (bystander suppression). Blocking/competition of major histocompatibility complex binding, immune deviation, anergy, and bystander suppression may not be mutually exclusive, and several mechanisms may be operative for a given altered peptide ligand. **Abbreviations:** APC, antigen-presenting cells; IFN, interferon; IL, interleukin; TCR, T-cell receptor; Th, T helper cells.
A number of APL have been developed based on the native structure of several candidate autoantigens in MS. Among them, only NBI-5788 has been tested for clinical efficacy in MS patients (3–5). NBI-5788 is an APL derived autoantigen from an immunodominant region of native myelin basic protein (MBP) 83–99, with a four amino acid substitution important for T-cell recognition. The chemical name for NBI-5788 is (D-Ala83, Lys84, Leu89, Ala91) MBP (83–99) NH2 (6). Two amino acid substitutions are relevant to biological activity, one at position 91 (lysine to alanine) and the other at position 89 (phenylalanine to leucine); these substitutions result in a nonstimulatory peptide analog that binds to the TCR of MBP (83–99)-reactive Th1 cells (6).

**Altered Peptide Ligand and Experimental Autoimmune Encephalomyelitis**

MS is an inflammatory disease mediated by autoreactive T-cells that recognize neuroantigens in the central nervous system (CNS). MS and its laboratory animal counterpart, EAE, provide precedents demonstrating that T-cells sensitized to myelin antigens produce inflammatory demyelinating diseases of the CNS. EAE can be induced by immunization of animals with neuroantigens or by adoptive transfer of CD4+ T-cells reactive to immunodominant regions of myelin antigens (2,3). EAE induced with myelin proteolipid protein (PLP) peptide 139–151 is known to be mediated by Th1 cells that recognize tryptophan 144 as the primary TCR contact point. An APL generated by a single amino acid substitution (tryptophan to glutamine) at position 144 (Q144) can inhibit the development of EAE induced with the native PLP (139-151) peptide (W144) (7). This APL induces T-cells that are cross-reactive with the native peptide, which produce Th2 cytokines (interleukin IL-4 and IL-10) as well as Th0 (interferon IFNγ, and IL-10) cytokines. Adoptive transfer of T-cell lines generated with the APL confers protection from EAE (7). Similarly, stimulation of polyclonal myelin oligodendrocyte glycoprotein (MOG) 35–55-specific T-cells with an MHC variant peptide results in the induction of anergy, as defined by a dramatic reduction in proliferation and IL-2 production upon challenge with the wild-type peptide (8). Furthermore, treatment of T-cell lines with this peptide in vitro significantly reduces their encephalitogenicity upon adoptive transfer.

NBI-5788 is an APL derived autoantigen from an immunodominant region of native MBP (83–99). NBI-5788 decreases both the incidence and severity of disease in models of acute EAE (Lewis rat) and of chronic/progressive EAE (SJL mouse) (7). Although the mechanism of action of NBI-5788 is still not known, available data suggest that it may act via generation of NBI-5788-specific Th2 cells regulating pathogenic Th1 cells through cytokine production (4). Furthermore, by targeting MBP-autoreactive T-cells, NBI-5788 may downregulate the MBP-specific myelin-damaging immune response.

Two recent studies aimed at optimal binding of APL with HLA-DR2 aroused new interest for enhancing the tolerogenic efficacy of APL (9,10). On the basis of binding motif of MBP (83–99) to HLA-DR2, an APL with modified amino acid composition was developed with the hope of suppressing MS more effectively. The enhanced efficacy of these APLs in EAE induced in SJL/J mice with PLP (139–151) was demonstrated. During that treatment protocol, the administration of APLs (9,10) after the onset of disease led to stasis of its progression and suppression of histopathological evidence of EAE. The mechanisms by which these effects are achieved have been examined in several types of assays: binding of APLs to
I-A(s) in competition with PLP (139–151) (blocking), cytokine production by T-cells (Th2 polarization), and transfer of protection by CD3(+) splenocytes or, notably, by APL-specific T-cell lines (induction of regulatory T-cells) (9,10).

These data, from studies of EAE, show that changing a single amino acid in an antigenic peptide may influence T-cell differentiation and suggest that immune deviation may be one of the mechanisms by which APL can inhibit an autoimmune disease. However, the wide heterogeneity of responses to multiple myelin antigens in human populations still poses a significant challenge to the use of APL in MS patients (11).

Clinical Experience with APL of MBP 83–99

The results of two clinical trials testing the ability of MBP APL to reduce the number of MS lesions were published simultaneously in *Nature Medicine* in 2000. Kappos et al. (4) undertook a double-blind, placebo-controlled study involving 142 patients and tested three different doses of the APL (5, 20, and 50 mg) in weekly subcutaneous injections. The authors did not find a difference in the relapse rate between APL- or placebo-treated patients, but reported that the volume and number of enhancing lesions were reduced in patients receiving the lowest APL dosage. Although no worsening of the clinical course was found, this trial was terminated because of a high incidence of immediate-type hypersensitivity reactions, which occurred mostly at the 50 mg dose. After a study of eight patients, Bielekova et al. (5) reported that APL treatment led to a higher incidence of MS exacerbation in three patients and, in two of them, linked that escalation to APL treatment. Those immunological studies suggested an encephalitogenic potential for MBP peptide (83–99) in a subgroup of patients. The results of both trials appeared similar in which neither study demonstrated a statistically significant difference between treated and untreated patients nor baseline versus treatment with respect to clinical manifestations. Furthermore, there was improvement on magnetic resonance imaging (MRI) in the placebo-controlled, double-blinded study by Kappos et al. (4).

Alteration of Autoimmune Responses in MBP APL-Treated MS Patients

APL studies in EAE have suggested several mechanisms that may underlie the mode of APL’s action. These include influences on the pathogenic T-cell population at the level of TCR (partial agonist, antagonism, and T-cell anergy), and induction of the regulatory T-cells specific for APL. The latter can produce anti-inflammatory cytokines after cross-activation with autoantigen, i.e., bystander suppression (Fig. 1). In MS patients who were treated with NBI-5788 MBP APL, NBI-5788-reactive T-cell lines exhibited an increased frequency of cross-reactivity with MBP (83–99) (12). Cytokine secretion by APL-reactive T-cell lines from NBI-5788-treated MS patients is more frequently Th2-like compared with T-cell lines from untreated MS patients (13). On the contrary, Bielekova et al. (5) reported that the APL-reactive T-cells, in their treated MS patients, were Th1 biased. Currently, there is no explanation for this apparent discrepancy; however, the different APL dosages used in the two clinical trials may, at least in part, account for the difference. In another study, a 4- to 16-week course of APL therapy induced a persistent (2–4.5 years) increase in the frequency of T-cell responses to both APL and the native MBP in a portion of MS patients so-treated (12).

In assessing these findings, it is important to appreciate the fact that attempting to associate Th1 versus Th2 cells with pathogenic or beneficial clinical effects is
dangerous and sometimes contrary to the truth. Frequently, Th2 cells have been identified as pathogenic in conditions that are believed to be Th1 mediated, e.g., type 1 diabetes (14) and some EAE models (15). Moreover, the pathogenesis of MS is extremely diverse. For example, antibody-mediated mechanisms of tissue damage have been described in patients with MS, and immune deviation toward the Th2 phenotype of cytokines may alter pathogenic antibody responses and possibly exacerbate MS, as demonstrated in the EAE of marmosets (15).

New Clinical Trials Using NBI-5788

Like T-cells, which do not respond in an “all-or-none” mode to cognate or altered ligands, APL therapy is not conclusively good or bad judging from current studies. These studies suggest that there may be significant limitations to peptide based therapies in an outbred species such as humans. However, observations regarding the clinical effects of APL should stimulate further research into which patients are most likely to benefit from such therapy. To this end, a randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, and efficacy of NBI-5788 in patients with relapsing MS is currently under way. This study will be conducted at approximately 25 medical centers in the United States and Canada. The rationale for this broad coverage is based on exploratory analysis of a phase II study in 142 patients with relapsing–remitting MS who were treated with placebo or with 5, 20, or 50 mg of NBI-5788. Several secondary efficacy measures of these groups, including MRI, indicated that this treatment was beneficial for recipients of the 5-mg dose (4) to a statistically significant extent compared with the placebo. That is, after 12 and 16 weeks, the treated group had a reduction from baseline in the total volume of gadolinium (Gd)-enhancing lesions. However, because these results were not adjusted for multiple analysis, they provide only suggestive evidence of treatment benefit that should be confirmed in an additional study.

ARE STATINS A TREATMENT OPTION FOR MS?

Introduction

The family of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors, collectively known as statins, is used clinically to reduce cholesterol levels in patients. The enzyme HMG-CoA reductase catalyzes the conversion of HMG-CoA to L-mevalonate (16,17). By its inhibition, statins prevent biological activities downstream of L-mevalonate. Currently, statins are the most effective agents available for the treatment of high blood cholesterol levels. An overwhelming amount of evidence confirms that statins decrease cardiovascular-related morbidity and mortality in individuals with and without coronary artery disease (16,17).

Lovastatin was the first of these medications to be introduced in the United States, and since then statins have become established as safe and well-tolerated drugs (18). Infrequent side effects include a dose-dependent elevation of hepatic transaminases (2%) and a dose-independent myopathy (0.1–0.5%). The latter adverse effects may result from a coenzyme Q (10) (CoQ) deficiency because inhibition of cholesterol biosynthesis also inhibits the synthesis of CoQ (17,19).

Atorvastatin is a widely prescribed statin and has a favorable safety profile compared with other statin drugs currently available (20). Adverse reactions have usually been mild and transient. Uncomplicated myalgia is reported in up to 5% of patients taking atorvastatin (19–21). Myopathy, defined as muscle ache or muscle weakness in conjunction with increased creatine phosphokinase (CPK)
values (>10 times the upper limit of normal), should be considered in any patient with diffuse myalgia, muscle tenderness or weakness, and/or marked elevation of CPK. The risk of myopathy increases with concurrent administration of cyclosporine, fibric acid derivatives, erythromycin, niacin, azole antifungal preparations; these medications are not permitted in this trial.

Summary of Preclinical Experience

Stanislaus et al. (22) conducted the first study to examine the effect of statin on EAE in Lewis rats. The authors reported the downregulation of inflammatory mediators [such as tumor necrosis factor (TNF)-α therapy inducible nitric oxide] in macrophage and glial cells in culture (23). Subsequently, two independent studies documented beneficial clinical effects of statins in murine models of MS. Aktas et al. (24) demonstrated that both subcutaneous and oral (1 mg to 10 mg/kg) administration of atorvastatin inhibited the development of actively induced chronic EAE in SJL/J mice and significantly reduced inflammatory infiltration into the CNS. When treatment was started after disease onset, atorvastatin reduced the incidence of relapses and protected recipients from the development of further disability. Both the reduced autoreactive T-cell response measured by decreased proliferation upon exposure to the encephalitogenic peptide PLP (139-151) and the cytokine profile indicate a potent blockade of the Th1 immune response. In vitro, atorvastatin not only inhibited antigen-specific responses but also decreased T-cell proliferation mediated by direct TCR engagement independent of MHC class II and lymphocyte function-associated antigen-1 (LFA-1). Inhibition of proliferation did not rely on apoptosis induction but was, instead, linked to a negative regulation of cell cycle progression. However, early T-cell activation was unaffected, as reflected by unaltered calcium fluxes.

Several murine models of EAE have been used by Youssef et al. (25) to evaluate the effects of atorvastatin. These models include MOG (35-55) peptide-induced EAE, PLP (139-151)-induced EAE in SJL/J mice, and MBP Acl-11-specific TCR transgenic mice. The authors showed that atorvastatin at 1 and 10 mg/kg doses could prevent or reverse ongoing relapsing paralysis in EAE. This clinical result was associated with a reduction in histological signs of EAE, reduced MHC class II expression on microglia in vivo, and suppression of IFN-γ-inducible class II expression on microglia tested in vitro. Also, when atorvastatin treatment was discontinued, mice did not develop EAE. Treatment in vivo or in vitro with atorvastatin suppressed CNS autoantigen-specific T-cell proliferative responses in a dose-dependent manner. Additionally, atorvastatin treatment of adult mice in vivo induced a Th2 bias, and treatment of naive CNS autoantigen-specific Th0 cells promoted differentiation of Th2 cells. In vivo as well as in vitro, atorvastatin induced a Th2-biased cytokine response, as evident by a reduction in Th1 cytokines including IFN-γ, IL-2, IL-12, and TNF-α, and an increase in secretion of Th2 cytokines including IL-4, IL-5, IL-10 and transforming growth factor (TGF)-β. The use of these atorvastatin-induced CNS autoantigen-specific Th2 regulatory cells as donor cells protected naïve recipient mice from EAE induction. Used in a clinically approved equivalent dose (wt/wt comparison), atorvastatin can reverse ongoing relapsing paralysis and induce regulatory Th2 cells that may mediate protection from EAE in vivo. Treatment withdrawal experiments, as well as adoptive transfer studies, indicate that there may be a sustained immunoregulatory effect. Adoptive transfer studies imply that tolerance is mediated by induction of regulatory cells (suppression). These studies do not preclude the possibilities that other mechanisms (i.e., anergy or deletion) could also be involved to some extent.
To identify potential targets on cells of the immune system where statins might act, Nath et al. (26) administered lovastatin and induced the expression of GATA3 and the phosphorylation of STAT6. However, lovastatin inhibited tyrosine phosphorylation of Janus kinase 2, tyrosine kinase 2, and STAT4. Inhibition of the Janus kinase-STAT4 pathway by lovastatin modulated T0 to Th1 differentiation and reduced cytokine (IFNγ, and TNFα) production, thus inducing Th2 cytokines (IL-4, IL-5, and IL-10). Also inhibited were T-bet (T box transcription factor) and NF-κB in activated T-cells. Lovastatin significantly reduced the infiltration of CD4- and MHC class II-positive cells into the CNS. Further, it stabilized IL-4 production and GATA-3 expression in differentiated Th2 cells, whereas in differentiated Th1 cells, it inhibited the expression of T-bet and reduced the production of IFNγ. Affymetrix DNA microarrays demonstrated a significant change in the expression of about 158 immune system-related genes (including 127 genes reported earlier) in lovastatin-treated versus untreated EAE, of which 140 genes were suppressed and only 18 genes were upregulated. These altered genes encode leukocyte-specific markers and receptors, histocompatibility complex molecules, cytokines/receptors, chemokines/receptors, adhesion molecules, components of the complement cascade, cellular activation and transcription factors, and signal transduction-related molecules. Interestingly, Th2 phenotype cytokines, such as IL-4, IL-10, and TGF-β1 and transcription factors such as peroxisome proliferator-activated receptor-γ, were upregulated by lovastatin treatment as further revealed by real-time polymerase chain reaction and immunoblotting (27). The outcomes of these studies suggest a hypothetical view of the mechanism of action of statins in MS (Fig. 2).

Glatiramer acetate (GA) is a random synthetic copolymer, efficacious in reducing demyelination-associated exacerbations in patients with relapsing–remitting MS and in several EAE models. The high affinity of GA for MHC grooves or the uptake of GA by antigen-presenting cells leads to presentation of GA-specific cells that are Th2 biased. Clearly, this favored mechanism of action by GA differs from that of statin, which may involve multiple components of the immune system. GA is only partially effective in MS. However, a combination of medications with distinct immunomodulatory mechanisms may enhance the efficacy of individual agents in treating MS. Stuve et al. (29) conducted a study to examine the efficacy of atorvastatin–GA-combination therapy in EAE. Suboptimal doses of atorvastatin and GA were determined, after which the clinical efficacy of combining these suboptimal doses was found to have a synergistically beneficial effect in suppressing clinical signs of EAE. Yet, the administration of atorvastatin or GA alone at suboptimal doses did not suppress antigen-specific T-cell proliferation in vivo. Moreover, the combination was as effective as single agent at optimal doses. Similarly, combination treatment using atorvastatin and GA at suboptimal doses was associated with enhanced lymphocyte secretion of IL-4 and IL-10 and TGFβ and decreased secretion of IL-2 and IFNγ. Histopathological examination showed decreased infiltration of blood leukocytes into CNS tissue in animals treated with this combination therapy. These results provide a rationale for testing the combination of atorvastatin and GA in patients with MS.

Clinical Experience with Statins in MS

Considerable data indicate that statins affect innate immune responses, manifested as endothelial cell activation and as macrophage, natural killer cell, and neutrophil effector function (18). Similarly, statins exert effects on acquired immune responses
via suppression of antigen presentation and T-cell activation in vitro and in vivo. MS is believed to be a Th1-mediated autoimmune disease, with activated CD4+ T-cells playing a central role. Humoral immune responses have also been implicated in the pathogenesis of MS. The presence of MS lesions surrounding post capillary in the brain venules suggests that extravasating systemic immune cells participate in plaque formation, axonal damage, and neurological disability. Statins may influence myelin-reactive T-cell reactivation and expansion in the periphery and promote...
inflammation and autoimmunity within the CNS of individuals with MS (Fig. 2).
Indeed, preclinical studies in numerous rodent models of EAE suggest that statins
decrease the migration of leukocytes into the CNS, inhibit MHC class II and costi-
mulatory signals on antigen-presenting cells, decrease the expression of inflamma-
tory mediators by T-lymphocytes, and lower the amount of inflammatory
mediators in the CNS. These immune regulatory properties of statins imply that they
may be beneficial in the treatment of MS.

In 2003, Sena et al. (30) reported the results of an observational study of seven
female patients who had relapsing–remitting MS after one year of monotherapy with
40 mg of lovastatin. The authors noted a reduction in the mean number of Gd-
enhancing lesions but no great difference between pretreatment and treatment
expanded disability status score (EDSS). Three of these patients remained free from
relapses, and the mean annual relapse rate decreased during the year. However, new
lesions appeared on T2-weighted images in five of the patients as the study ended.

In a multicenter, open-label, single-arm phase II study to evaluate the safety
and efficacy of simvastatin, 30 patients with relapsing–remitting MS (31) were eli-
gible for the drug after each manifested at least one Gd-enhancing lesion detected
by three monthly MRI scans obtained while off-therapy. These subjects received
80 mg of simvastatin per day orally for six months. The primary efficacy outcome
was the mean number of total Gd-enhancing lesions present at months four, five,
and six of therapy in comparison to those at the three-monthly MRI scans obtained
at baseline.

The result was a marked decrease in the number of Gd-enhancing lesions (\(P <
0.0001\)), with a similar decrease in the total volume of Gd-enhancing lesions
(\(P < 0.002\)). The mean differences in the EDSS, multiple sclerosis functional composite
z-scores, and performance scale scores, between baseline and month 6 of treatment, were
not found to be significantly different from zero for any of the scores. The Spearman
rank correlations did not indicate a moderate or high correlation among the MRI out-
comes and neurological assessment scores. Four relapses were confirmed during the pre-
treatment phase. A total of five confirmed relapses were reported during the treatment
phase, one of which involved a subject who experienced a relapse during the
pretreatment phase. The annualized relapse rate for treated subjects during the pretreat-
ment and treatment phases were 0.43 and 0.38, respectively (\(P \geq 0.9999\)). No serious
adverse events were reported during the treatment phase. Expected adverse events
due to study medication, were elevated creatine kinase values, elevated liver function
tests, and muscle pain or weakness.

This study design involved a cross-over trial in which subjects were observed
clinically by MRI, the most sensitive technique for visualization of MS lesions. Cran-
ial MRI scans demonstrated significant decreases in both the number and volume
of Gd-enhancing lesions (Table 1), which is consistent with the proposed mechanism
of action as described above. Indeed, Gd-enhancement, as measured by MRI, in
patients with MS is known to be particularly sensitive to therapies that inhibit T-cell
activation, T-cell–endothelial adhesion via integrins, and matrix metalloproteinase
activity. The lack of effect on relapse rate was expected, given the small size of the
cohort and short duration of our study. Larger, placebo-controlled studies should
assess the clinical effect.

Significant variability was documented for cytokines measured over the dura-
tion of the trial; however, the ratio between representative Th1 versus Th2: IL-4
versus IFN-\(\gamma\) cytokines showed a trend favoring Th2 cytokine production during
treatment in comparison to baseline measurements (\(P = 0.007\)) (Table 1). Results
from the cohort indicated that treatment with simvastatin did not affect relative numbers of monocyte (CD14+) and lymphocyte (CD3+, CD4+, CD8+, and CD19+) subsets. Analysis of activation and costimulatory markers revealed that simvastatin decreased CCR5 expression on lymphocytes (P < 0.01) and CD86 expression on monocytes (P = 0.02).

Rationale for Large-Scale Clinical Trials Using Atorvastatin

Since 1993, six disease-modifying agents have been approved for relapsing–remitting MS: IFN β-1a (Avonex® and Rebif®), IFN β-1b (Betaseron®), GA (Copaxone®), Mitoxantrone (Novantrone®), and Natalizumab (Tysabri® bring reevaluated by FDA due to PML). Nevertheless, the limited effectiveness of these treatments as well as the inconvenience and toxicity associated with their use emphasize the need for new therapies. The results of two clinical studies (30,31) provide hope, yet the number of patients and the design of the study do not allow for a definitive conclusion on the role of statins in MS. The main concern of these two studies is whether, without a placebo group, the reduction in disease activity as measured with MRI could be caused by regression to the mean (31). As Polman and Killestein (32) pointed out, the inclusion of patients in the study because of Gd-enhancement might have unintentionally selected those with active disease who would subsequently undergo reductions in disease activity with or without intervention.

Considering these facts, a randomized, double-blind, placebo-controlled, multicenter study is under way to evaluate the efficacy and safety of atorvastatin in patients with a clinically isolatable syndrome and high risk of conversion to...
MS. Atorvastatin is a widely prescribed statin and has a favorable safety profile compared with the other statin drugs currently available. The primary objective of this study is to evaluate the ability of atorvastatin versus a placebo to decrease or delay MS that is observable either clinically or via MRI. The preliminary effects of atorvastatin on measures of brain atrophy and immunologic parameters will also be evaluated.

The clinically beneficial effects of statin, evidently, are not limited only to MS. For example, the results of a double-blind, randomized, placebo-controlled trial in 116 patients with rheumatoid arthritis (33) showed that statin mediated modest but clinically apparent anti-inflammatory effects and modified vascular risk factors in the context of high-grade autoimmune inflammation. Additionally, statin use in individuals 50 years of age and older has been associated with up to 70% decrease of dementia (13). Presumably this effect does not result from the lipid-lowering effects of statins, since subjects treated with nonstatin lipid-lowering agents had no such reduction in dementia. Another cohort study (34) demonstrated that the use of statins decreased the risk of Alzheimer’s disease in subjects younger than 80 years. The broad effects of statins on innate and adaptive immune systems are overall suppression of the immune system, somewhat reminiscent of steroid’s effects. While making use of this immune suppressive property to treat autoimmune diseases, one must include attention to the side effects specific to immune system. Continued basic and clinical studies will reveal statin-sensitive pathways that may offer further opportunities for the generation of novel disease-modifying drugs to treat MS and other chronic inflammatory diseases.

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INTRODUCTION

Several lines of evidence suggest that a sequence of environmental triggers upset the delicate balance between tolerance and nontolerance of “self” antigens, but the environmental triggers and the detailed immunogenetic predisposing factors are unknown. Though the exact etiopathogenesis of multiple sclerosis (MS) is unknown, the leading theory suggests that T-cells are the conductors of a misdirected immune response that targets myelin in the central nervous system (CNS). They recruit many other components of the innate and adaptive immune system, producing the inflammation seen pathologically in the CNS (1,2). Subsequently, epitope spreading and immunological memory develop and give rise to the chronicity of the disease.

Though the actual cause of the axonal degeneration and neuronal dropout that characterize progressive MS still remains in question, evidence continues to point to the early phases of disease in which CNS inflammation dominates (Fig. 1). Brain lesions examined at this stage show the greatest number of pathologically evident axonal transactions (3) and neurons tend to express amyloid precursor protein, a marker of imminent cell death (4). The theory that inflammation brings about these neurodegenerative changes is supported by clinical experience. Numerous studies demonstrate that immunosuppressive treatments capable of reducing inflammation are able to stabilize or retard the development of further disability in patients with MS. This is particularly true for patients in whom inflammation is evident either by the continued presence of clinical relapses or gadolinium (Gd) enhancement on magnetic resonance imaging (MRI) studies. In this chapter, we will consider the evidence that supports the use of the synthetic nitrogen mustard-like molecule cyclophosphamide (CTX), first as an immunosuppressant by itself, then as part of a complete immunoablation regimen requiring rescue with autologous stem cell transplants.

Immunomodulatory therapy, with interferon or other agents, is the first line treatment for most cases of MS, but immunosuppressant treatments are considered once these fail. Some patients demonstrate an aggressive course from the onset of their illness and warrant initial immunosuppression therapy. This review first considers
the escalation of therapy from immunomodulatory treatment to CTX. The use of mitoxantrone, another immunosuppressant, is covered elsewhere in this text. Subsequently, we rationalize why complete immunoablation followed by a “rescue” using an autologous stem cell transplant might be an option for some patients. Since immunomodulators are thought to work for the most part on the immune system outside the CNS, the ability of immunosuppressants such as CTX to cross into the CNS via the blood–cerebrospinal fluid (CSF) barrier (5,6) makes them ideally suited for dealing directly with the inflammatory response attacking the nervous system.

CYCLOPHOSPHAMIDE

Though many immunosuppressants have been tried in the treatment of patients with progressive MS, no single agent has been explored more than CTX in the past three decades. CTX is used in the treatment of a number of neoplastic and autoimmune illnesses. It can be administered orally or intravenously in a variety of dosing schedules. The toxic effects of this agent are well known, dose-dependent, and generally tolerable in an outpatient setting. A Cochrane review could not make any definite conclusion regarding its overall efficacy in MS, but this has to do with the lack of large properly controlled studies with proper patient selection (7). Interest in the drug and its continued use revolve around a series of smaller uncontrolled studies in which clear stabilization has ensued, in patients either failing immunomodulatory treatments or who have a more aggressive course from the outset (8,9). One of the first studies to support the use of CTX was a study in which patients were followed after being given a single pulse of agent (10). Patients who showed initial

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**Figure 1** The course of multiple sclerosis and the substrate of progression. At early stages of multiple sclerosis, inflammatory activity, manifested clinically by relapses and by magnetic resonance imaging as newly appearing or enhancing lesions, predominates. At later stages a neurodegenerative process becomes more prominent, manifested clinically by slow progression and by magnetic resonance imaging as progressive atrophy. At every stage, it is felt that the inflammatory events trigger or sustain the neurodegenerative process. *Abbreviations:* MS, multiple sclerosis; MRI, magnetic resonance imaging.
stabilization tended to progress again after the first year of follow-up, prompting some to contend that boosters are required to maintain the response following the induction of stabilization (11). A number of patient and disease factors predict a positive response to treatment including younger age, relapsing–remitting (RR) MS, or shorter duration of secondary progressive (SR) MS, evidence of recent ongoing inflammation (clinical relapses or enhancing MRI lesions), and a more rapid progressive course (12). These factors fit with our current understanding of disease mechanisms and the perceived mode of action of this therapy that is targeting the inflammatory component of the disease. The effects of monthly intravenous CTX pulses on the inflammatory component of MS was best demonstrated by the rapid reduction in active enhancing lesions on serial monthly MRIs in patients followed clinically for more than two years (13). The latter study, unlike many of the other trials, used CTX exclusively, without the concurrent administration of a monthly steroid pulse. Studies of CTX that did not demonstrate a clinical effect either did not utilize pulse therapy or targeted patients who were in a progressive phase of MS with an absence of inflammation (14,15). Despite the general lack of evidence for a treatment effect in the progressive phase of MS, a more recent study suggests that CTX may exert a stabilizing effect even in pure progressors (16).

A number of different regimens have been used without a systematic comparison between them. While there is no set regimen, a reasonable approach to pulse CTX therapy, that lends itself to outpatient treatment, has been used successfully by the Harvard group and others. Rapid disease control is accomplished by a pulse of high dose methylprednisolone (MP, 1 g i.v. daily for five days). CTX (800 mg/m² intravenously) is given on the fourth day of the MP. Subsequent maintenance of disease response is achieved with boosters of CTX and MP (1 g i.v.) starting a month after the first dose. The dose of CTX is increased with each booster to produce a nadir leucopenia of 1500–2000/mm³. The total maximum single dose is suggested to be 1600 mg/m², but rarely do patients require more than 1400 mg/m² to obtain the desired nadir of leucopenia. This dose of CTX is then continued with MP every four weeks for the first year, then every six weeks for year 2 and finally every two months in year 3 (17). In this study, a clear stabilizing effect was found, particularly in younger patients that were still having relapses, despite the SPMS course of disease.

Patients who are experiencing increased disease activity while on disease modifying drugs (DMD) might also benefit from short pulses of CTX, in an effort to stabilize the MS and reinduce a response to the DMD. Numerous but small studies have reported various degrees of disease stabilization of these “breakthrough” patients (18,19). One study randomized 59 patients with significant disease activity despite treatment with β-interferon to receive either six monthly pulses of MP alone or in combination with CTX. Patients continued to receive concurrent treatment with interferon throughout the 24 months of the study (20). With 24 months of follow-up, the number of patients who reached a predetermined definition of “treatment failure” was halved in the patients who received the CTX. Significant improvements both in clinical and MRI evidence of disease activity occurred when CTX was added to the treatment. This suggests that CTX can act as “rescue therapy” for patients faltering on β-interferon therapy. In other patients who may be in “transition” between the RR and SP phase of their illness, but in whom relapses are still evident, CTX has been shown to slow progression (21).

The toxicity of conventional doses of CTX (22) involves nausea or vomiting during and in the first few days following administration of the drug. This can be controlled with preventative antiemetic medications. Dose-dependent neutropenia
is short-lived. The nadir of the leukocyte count will occur in the second or third week following chemotherapy. It is rarely associated with febrile neutropenia or sepsis at the doses administered for the treatment of autoimmune diseases. At monthly doses of greater than 750 mg/m², patients may develop alopecia—this begins two or three weeks following drug administration—but will reverse in the months following discontinuation of the medication. There is a possibility of premature ovarian failure with the induction of infertility or menopause, especially in patients receiving cumulative doses > 300 mg/kg (23,24). Males may develop decreased sperm counts and infertility, but this may resolve following discontinuation of the drug (25,26). Successful pregnancies have been documented in patients that have been treated even with high-dose CTX (27). CTX is teratogenic (28,29) and patients must use contraception during the time of treatment. Somewhat unique to CTX has been the possibility of hemorrhagic cystitis due to the concentration of this drug’s metabolites in the bladder (30). Good hydration during administration and frequent voiding following administration usually avoids this problem. Concurrent administration of Mesna, a drug that specifically neutralizes CTX’s urotoxic metabolic, is probably not warranted at the doses used for monthly pulses, though its use has been advocated in at least one study (31). There is an increased risk of developing a secondary cancer or leukemia. The risk appears to peak five to six years after treatment and is about 2 to 20 times the risk of age-matched persons not exposed to chemotherapy (32,33). The peak risk of secondary cancers tends to occur later than for leukemia. The risk of a secondary malignancy is dose-dependent and the recommendation is not to exceed a total cumulative lifetime dosage of 80 to 100 g (34). If a large individual (2 m²) requires the maximum dosage of 1400 mg/m² using the regimen of CTX described above, the total cumulative dose is only ~72 g.

The pharmacology of CTX is well understood (22). The drug requires cellular enzymatic conversion to an active form that then reacts with many different molecules in the cell. Ultimately, alkylation of DNA disrupts cellular function and results in cell death. CTX induced apoptosis modulates many aspects of the immune response (35,36). The mechanism of CTX stabilization in MS is unknown, but it probably acts through more than its antiproliferative properties, and considerable evidence attests to its ability to act as an immunomodulator (37). In MS, the immune-mediated reactions are thought to be due to a predominant Th1-directed response. Similar to the current DMD, CTX steers immune reactions toward Th2 responses, but it also downregulates IL-12, a prime cytokine involved in the kick-start of any Th1-mediated immune reaction (38) as well as inducing CCR4+ cells that released high levels of IL-4 further downregulating Th1 responses (39).

COMPLETE IMMUNOABLATION AND AUTOLOGOUS STEM CELL TRANSPLANTATION

Disease modifying agents that curtail the flow of inflammatory immune cells into the brain reduce both clinical and MRI-linked inflammatory events and slow, at least in the short term, clinical progression. The capacity to modify the course of MS appears to be related to the intensity of immune suppression. Inevitably, progression resumes because incomplete suppression of inflammation fails to halt the loss of axons and neurons. More intense immune suppression can regain control of the inflammatory responses, but some degree of CNS destruction has occurred. Complete abrogation of the inflammatory response before there is permanent neurologic damage, at an
early stage of MS, may be more successful at preventing the inevitable progression and ultimately may set the stage for restorative repair processes.

**TRANSPLANT STUDIES IN MS**

Current immunosuppressive treatments, using lymphocytotoxic drugs or biologicals, have in common the ability to reduce, but not eliminate the autoreactive immune system. MS inevitably continues because of residual CNS inflammation or persistent “memory” of myelin attack. High-dose chemotherapy, used for the treatment of leukemia in the context of allogeneic bone marrow transplantation, induces an intense immunosuppression. Adapting this procedure to patients with autoimmune diseases such as MS has produced promising results.

Animal studies, studies of humans with autoimmune diseases undergoing transplantation for malignancy, and autologous stem cell transplantation (ASCT) for patients with other autoimmune diseases provide the rationale for using this treatment in MS. Experimental allergic encephalitis (EAE), an MS-like disease induced in rodents by immunization with spinal cord homogenates or myelin proteins resolves following treatment with total body irradiation or high-dose CTX and transplantation of marrow from a healthy littermate (40–42). Transplantation at an early time-point has been shown to prevent glial scarring while late transplantation may moderate, but not prevent glial scarring (43), highlighting the benefit of early intervention before the illness transforms from an inflammatory state to a progressive neurodegenerative disease. Although occasional relapses (44,45) have been reported, significant durable remissions of autoimmune diseases (46), including MS (18,20,47), have been documented in patients that have undergone hematopoietic stem cell transplantation (HSCT) for a concurrent malignancy. Finally, promising results have been reported in phase I–II autologous stem cell transplant studies of patients with advanced and refractory systemic lupus erythematosus (48), scleroderma (49), rheumatoid arthritis (50), and juvenile rheumatoid arthritis (51,52).

Worldwide, more than 500 patients have undergone ASCT for an array of autoimmune diseases (53) with more than 150 MS patients treated in this manner (Table 1). All patients receive high-dose cytotoxic chemotherapy as a preparative regimen immediately prior to transplantation. TBI or cytotoxic antilymphocyte antibodies have been added to enhance the destruction of the immune system. Stem cell grafts that may be depleted of lymphocytes are infused following the cytotoxic regimen. The adult hematopoietic stem cells (HSC) in these grafts give rise to cells that repopulate the endothelium, the blood, and the immune system. HSC that differentiate into the lymphocyte lineages give rise to a naïve polyclonal immune system with a diverse protective repertoire. HSC do not carry the immunologic memory of previous exposures, and thus the regenerated immune system will not have a memory of its previous reactivity (and auto-reactivity). Given that the exact sequence of environmental triggers is unlikely to be repeated as the new immune system evolves, a reappearance of autoimmunity would be improbable.

While the first trials using transplantation for the treatment of MS concentrated on the safety of the procedure, information has been accumulating about the effectiveness of the procedure. More importantly, systematic comparisons of inter-patient variability in disease characteristics and transplant regimens are allowing investigators to make conclusions about the factors that determine positive treatment outcomes.
### Table 1  Summary of Patient Variables, Transplant Conditions, and Treatment Outcomes for Patients with MS Who Have Undergone Stem Cell Transplantation

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of pts.</th>
<th>Baseline EDSS</th>
<th>Duration of MS prior to SCT (months)</th>
<th>Graft selection</th>
<th>Conditioning regimen</th>
<th>Follow-up (months)</th>
<th>Outcome (number of pts positive/total number of pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nash et al. (54)</td>
<td>26</td>
<td>5.0–8.0</td>
<td>10–277</td>
<td>yes</td>
<td>CTX/TBI</td>
<td>yes</td>
<td>24</td>
</tr>
<tr>
<td>Burt et al. (55)</td>
<td>21</td>
<td>3.0–8.5</td>
<td>9–216</td>
<td>yes</td>
<td>CTX/TBI</td>
<td>no</td>
<td>24</td>
</tr>
<tr>
<td>Carreras et al., Saiz et al. (56,57)</td>
<td>15</td>
<td>4.5–6.5</td>
<td>12–228</td>
<td>yes</td>
<td>BCNU/CTX</td>
<td>yes</td>
<td>36</td>
</tr>
<tr>
<td>Rossiev et al. (58)</td>
<td>23</td>
<td>—</td>
<td>26–144</td>
<td>no</td>
<td>BEAM/Flud/Mel</td>
<td>yes</td>
<td>—</td>
</tr>
<tr>
<td>Kozak et al. (59,68)</td>
<td>7</td>
<td>6.5–7.5</td>
<td>48–168</td>
<td>yes</td>
<td>BEAM</td>
<td>yes</td>
<td>9</td>
</tr>
<tr>
<td>Openshaw et al. (60)</td>
<td>4</td>
<td>5.5–7.5</td>
<td>60–108</td>
<td>yes</td>
<td>Bu/CTX</td>
<td>yes</td>
<td>20</td>
</tr>
<tr>
<td>Fassas et al. (61)</td>
<td>15</td>
<td>4.5–8.0</td>
<td>24–336</td>
<td>no</td>
<td>BEAM</td>
<td>yes</td>
<td>40</td>
</tr>
<tr>
<td>Manacardi et al. (62,63)</td>
<td>10</td>
<td>5.5–8.0</td>
<td>72–228</td>
<td>no</td>
<td>BEAM</td>
<td>yes</td>
<td>15</td>
</tr>
<tr>
<td>Sun et al. (64)</td>
<td>4</td>
<td>6.0–7.0</td>
<td>84–228</td>
<td>yes</td>
<td>CTX/TBI</td>
<td>yes</td>
<td>12</td>
</tr>
<tr>
<td>Canadian Trial (Atkins HA, Freedman MS, unpublished work, 2005)</td>
<td>11</td>
<td>4.0–6.0</td>
<td>43–125</td>
<td>yes</td>
<td>Bu/CTX</td>
<td>yes</td>
<td>25</td>
</tr>
</tbody>
</table>

**Abbreviations:** ATG, anti-thymocyte globulin; BCNU, Carmustine; BEAM, BCNU, Etoposide, Cytarabine and Melphalan; Bu, busulphan; CTX, cyclophosphamide; EDSS, expanded disability status scale; Flud, Fludarabine; Mel, Melphalan; MRI, magnetic resonance imaging; SCT, stem cell transplantation; TBI, total body irradiation.
STEM CELL TRANSPLANTATION

HSCT is a complex procedure (Fig. 2) that includes:

1. Collection of a graft containing HSC.
2. Treatment of the underlying disease with high-dose cytotoxic therapy (the preparative regimen) resulting in the ablation of the bone marrow and immune system.
3. Infusion of the HSC graft that reseeds the marrow, moderating the myelotoxicity and providing for immune recovery.

Each aspect of the transplant can be performed in several ways and the exact method will influence its overall outcome. Regimen related complications and

Figure 2  Autologous stem cell transplantation for multiple sclerosis. Patients with aggressive multiple sclerosis have hematopoietic stem cells harvested following their mobilization from the bone marrow into the circulation by a combination of chemotherapy and cytokine. The graft contains many circulating blood cells, including lymphocytes. About 1% of the graft cells are hematopoietic stem cells. The stem cell graft is processed to enrich the hematopoietic stem cells and to remove contaminating immune cells, thus avoiding reintroduction of autoreactive memory cells following transplantation. Purified hematopoietic stem cells do not have immunologic memory. The preparative regimen, consisting of intensive cytotoxic therapy, is administered to destroy the autoreactive immune system responsible for chronic central nervous system inflammation. The purified stem cells are transplanted into the patient. Their daughter cells grow and mature into all the cells of the blood and immune systems without recollection of pre-transplantation antigen exposures, thus regenerating the blood organ and a naïve, presumably tolerant, immune system. Abbreviations: HSC, hematopoietic stem cells; MS, multiple sclerosis.
mortality are related to a number of factors including the source of the stem cells, the intensity of the chemotherapeutic regimen employed, previous exposure to cytotoxic chemotherapy, age, and pre-existing organ dysfunction. For instance, while allogeneic stem cell transplantation has a 20% to 30% mortality due to graft-versus-host disease (GVHD), mortality following ASCT may be as low as 3% (65). Near-normal cardiac, pulmonary, renal, and hepatic functions are necessary to cope with the stress of fluid and electrolyte disturbances and sepsis that may occur. Improved supportive care has changed resource utilization allowing some centres to perform transplants in a day hospital setting (66,67). HSC may be collected from the bone marrow or from the circulation. Marrow harvest is performed by multiple aspirations through a hollow needle inserted percutaneously in the marrow cavity of the posterior iliac crests in the operating room under general or spinal anesthesia. The procedure causes short-lived postoperative pain. HSC are collected following their mobilization from the marrow into the peripheral blood by one to three leukopheresis using peripheral or central venous access (54,56,68). Peripheral blood stem cell collections contain more stem cells and more lymphocytes than bone marrow grafts, but are technically better suited to ex vivo graft modifications, such as HSC purification or T-cell depletion. Most investigators collect peripheral blood stem cells as the source of stem cells, only 6 of the 85 patients with MS reported to the European bone marrow transplantation (EBMTR) used bone marrow harvest as the source of stem cells (69). Because so few MS patients have received a bone marrow transplant, it is impossible to discern an effect between these different sources of stem cells on the outcome of transplantation.

HSC are mobilized from the bone marrow by disruption of their homing mechanisms (70). Recombinant granulocyte colony stimulating factor (G-CSF) results in a dose-dependent mobilization of HSC from the marrow into the circulation (71). The drug is well tolerated, but occasionally may cause transient headache, fatigue, bone pain, and splenic enlargement (72), which has resulted in splenic rupture in a small number of healthy donors (73–76). G-CSF has also been associated with anecdotal case reports of thrombosis (77,78). Cytokines have been associated with flares of autoimmunity in some patients (54,55,79). While most flares have been transient and reversible, in one instance a patient with a cervical cord plaque developed respiratory failure due to a flare of disease activity in the lesion (80). Investigators have abandoned the use of cytokines alone for mobilization of stem cells for patients with autoimmune diseases because of the risk of stimulating the autoimmune disease process.

Cytokine-induced activation of autoimmunity can be prevented by the concurrent use of chemotherapy or steroids. CTX and G-CSF has been used extensively for stem cell mobilization in patients with cancer. Mancardi et al. (62) and Kozak et al. (59,68) using CTX and G-CSF did not report any change in MS activity associated with the mobilization protocol. In our own series, MRI scan done seven days after the chemotherapy but during G-CSF administration show a diminution of Gd activity compared to baseline scans (81). CTX toxicity includes transient syndrome of inappropriate antidiuretic hormone secretion, gastrointestinal upset, moderate alopecia, hemorrhagic cystitis, and transient pancytopenia with an attendant risk of febrile neutropenia or sepsis, but these rarely become life-threatening.

Adoptive transfer of immunity by transplanted lymphocytes has been demonstrated in some (82–84) but not all (85) recipients of bone marrow from donors with autoimmune diseases. Thus, autologous stem cell grafts, containing autoreactive lymphocytes, could potentially reinitiate the autoimmune disease following...
transplantation. Immune cell mediated reactivity can be removed from a stem cell graft by lymphocyte depletion using ex vivo depletion strategies. Generally lymphocytes are removed by purification of HSC using immunomagnetic technology (86–88). Stem cell grafts are incubated with an anti-CD34 monoclonal antibody that is immobilized on a paramagnetic particle. The labeled HSC are retained in the magnetic field of a stem cell selector device while the remaining cells are washed away. The stem cells are washed and collected by releasing the magnetic field. Generally a 10,000 to 100,000-fold depletion of lymphocytes can be obtained using the positive selection procedures. Ex vivo selection technology has been used to remove unwanted immune cells in about two-thirds of the transplants for MS reported to the autoimmune disease working party registry of the EBMT. Five published studies have used lymphocyte depleted stem cell grafts (54–56,60,68). While unprocessed stem cell grafts contained upwards of $50 \times 10^6$ T-lymphocytes/kg (61), the residual number of immune cells in processed grafts was device dependent and ranged from 1 to $120 \times 10^4$ T-lymphocytes/kg. Fassas et al. (61) did not detect a difference between the frequencies of posttransplant MS progression for 15 patients who received an unmanipulated graft compared with 9 patients who received a CD34 selected graft. However, interpreting this result is difficult because the processing procedure used in this study was only partially effective in removing contaminating lymphocytes. Furthermore, the intensity of the pretransplant preparative regimen was unlikely to ablate the immune system; the patient’s residual immunity would mask any potential treatment benefit of removing immune cells from a stem cell graft. It has been difficult to determine the role of ex vivo lymphocyte depletion because of the many difference in both the success of the immune ablation and the rigor of the lymphocyte depletion of the stem cell.

A number of preparative regimens, adapted from the treatment of lymphoproliferative diseases, have been used with the goal of depleting or ablating the autoreactive immune system (Table 1). TBI and some systemically administered chemotherapy (Busulphan or Cytarabine) cross the blood–brain barrier attacking immune cells lodged in the CNS. Where the preparative regimen includes an agent that would penetrate into the CNS, a reduction or disappearance in CSF oligoclonal banding has been seen in about a quarter of the patients (54,60,64, Atkins HA, Freedman MS, Unpublished Work, 2005). Peritransplant administration of anti-thymocyte globulin (ATG) has been used in the majority of patients. This drug contributes to the immune depletion of the patient and may also kill reinfused lymphocytes from the graft.

There is a 2% to 10% mortality following autologous transplantation for malignancy that is dependent on the conditioning regimen, patient age, comorbidities, and performance status. The EBMTR reports a 4.7% mortality rate for MS patients due to treatment related complications (69). Causes of death in patients undergoing transplantation for MS include cardiac toxicity, sepsis, veno-occlusive disease (Atkins HA, Freedman MS, Unpublished Work, 2005), and EBV lymphoproliferative syndrome (89). Preparative regimens containing Busulphan or TBI are generally more intense and have higher reported transplant related mortality. There is no indication that MS patients have an unusual susceptibility to transplant related morbidity.

Fortunately, serious complications and deaths have been sporadic in the published studies of patients with MS. The common short-term toxicities of transplant preparative regimens are listed in Table 2, but their frequency and severity vary depending on particular regimen used. The noninfectious complications of the
transplants for MS have been on the whole of moderate severity. This may reflect the relatively small number of patients in each cohort, the cautious patient selection or the lack of comorbidities in this otherwise healthy young patient population. Because MS patients commonly have abnormal bladder dynamics, forced diuresis, urinary catheterization to maintain bladder drainage, and intravenous MESNA have all been used to reduce hemorrhagic cystitis (55). Urinary bladder catheterization results in a higher incidence of urinary tract infections than seen in other transplant populations (54). The incidence of febrile neutropenia and the spectrum of infections reported have been representative of other autologous transplant populations receiving similar conditioning regimens. G-CSF has generally been administered following transplantation to hasten neutrophil recovery and this typically occurs within two weeks of the transplant. Platelet recovery occurs shortly thereafter. The median length of hospital stay for MS patients receiving transplants was about 25 or 26 days, although some MS patients have required stays up to two months for treatment of transplant related complications (61,62,68).

Between one-fourth and three-fourth of MS patients develop an engraftment syndrome, characterized by noninfectious fevers, an erythematous maculopapular or follicular rash predominantly on the upper trunk, and fatigue at the time of neutrophil recovery (54,56). Pruritis, minor pulmonary symptoms, and mild eosinophilia may also occur. Engraftment syndrome is not unique to MS patients undergoing transplantation (91). Symptoms last for a week or two and may resolve spontaneously. Some patients require a short pulse of steroids for symptom control.

The late effects of the chemotherapy include premature gonadal failure, shingles, and an increased risk of secondary malignancies. Between 10% and 20% of transplant recipients with MS develop disseminated or localized Varicella syndromes. This is not out of keeping with other transplant populations. Somewhat more unusual are reports of CMV reactivation following autologous transplantation for MS. This event is rare following autologous peripheral blood stem cell transplantation for malignancies, but occurred in 3 of 10 patients in one series (62) and in 4 of 9 patients in another (54). The reactivation of CMV likely reflects the degree of immune ablation achieved by the conditioning regimens.

The preparative regimen destroys circulating lymphocytes and patients become lymphopenic. NK and B-cell subpopulations recover three to six months after transplantation, but CD4 cells may remain low for two years or more. Alterations in the

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Side-Effects of High-Dose Therapy in the Immediate Posttransplant Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Sepsis</td>
</tr>
<tr>
<td>Decrease concentration</td>
<td>Hemorrhagic cystitis</td>
</tr>
<tr>
<td>Anorexia</td>
<td>Neutropenic enterocolitis</td>
</tr>
<tr>
<td>10–15% loss in body weight</td>
<td>Multiorgan failure syndrome</td>
</tr>
<tr>
<td>Oral stomatitis</td>
<td>Liver, renal, respiratory failure</td>
</tr>
<tr>
<td>Nausea, vomiting</td>
<td>Venocclusive disease of the liver</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Capillary leak syndrome</td>
</tr>
<tr>
<td>Fluid and electrolyte disturbances</td>
<td>Engraftment syndrome (90)</td>
</tr>
<tr>
<td>Alopecia</td>
<td></td>
</tr>
<tr>
<td>Pancytopenia</td>
<td></td>
</tr>
<tr>
<td>Blood and platelet transfusions</td>
<td></td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td></td>
</tr>
</tbody>
</table>
cytokine production by mononuclear cells and matrix protein production have been reported. Understanding the changes in immunity following transplantation may allow more selective and less toxic methods of immune manipulation in MS.

**MS OUTCOMES FOLLOWING TRANSPLANTATION**

The impact of transplantation on MS activity has been examined using a number of endpoints including, progression of disabilities, subjective and objective relapses, MRI lesions, and changes in CSF oligoclonal bands. All the published studies have a short follow-up period, so caution must be taken interpreting the results until sufficient maturation of the outcomes has occurred. With median posttransplant follow-ups between 9 and 40 months in the different studies, as many as 40% of patients have progressed. Treatment related factors, as previously discussed, and disease related factors might explain the difference in freedom from disease progression among the various studies.

Some studies show a plateau in the time-to-progression curve after the early posttransplant period (57), but generally there is an increasing number of treatment failures with longer follow-up (54,55,61). The lack of a plateau on progression-free survival curves suggest that increasing the intensity of the immune suppression has delayed but not eliminated the underlying autoimmune process. This optimistically would suggest that in very high-risk patients the transplant has, to a certain degree, been able to control disease activity. The use of myelotoxic preparative regimens such as BCNU, etoposide, cytarabine, and melphalan (BEAM) or Carmustine (BCNU)/CTX tends to have a higher failure rate than the stronger myeloablative regimens using busulphan (Bu)/CTX or CTX/TBI. Whether the complete ablation of the autoreactive immune system with reconstitution of a naïve immune system will allow tolerance to be reestablished is the goal of the ongoing Canadian trial of stem cell transplantation for MS.

While there may be ongoing disease activity following low intensity preparative regimens, these regimens may still provide benefit, as the progression of disabilities has stopped for many patients. The event-free survival, defined as freedom from any progression or relapse, was quite low in the study by Fassas et al. (61) yet progression-free survival was reported as 76% with 3.7 years of posttransplant follow-up. A similar analysis performed by Saiz et al. (57) showed MS activity in 65% of the patients while nearly 85% of their patients are free from progression four years after the transplant. The relative stability of neurological function in face of ongoing relapses suggests that these transplants have “reset” the disease to an earlier phase.

MRI metrics that have been examined include the number of Gd enhancing lesions, T2 lesion number, total T2 lesion volume, and atrophy. Transplantation reduces or eliminates inflammation in the CNS and this translates into a marked reduction or absence of Gd enhancing lesions. T2 lesion load is reduced and new T2 lesions either do not form or appear at reduced frequency. Generally posttransplant Gd activity or increasing T2 lesion load appears in patients with clinical evidence of progressive disease (54,55). While transplantation had a profound effect on inflammatory activity, there was ongoing evidence of atrophy as assessed by changes in total brain volume (92), change in third ventricle diameter (55), and atrophy of the corpus callosum (91). Interestingly, atrophy is progressive despite a reduction in the total T2 lesion volume and the absence of inflammatory changes.
The MRI studies illustrate the impact of transplantation on reducing the inflammatory but not the neurodegenerative component of MS.

**PATIENT SELECTION**

Initial transplantation studies tended to select patients with advanced SPMS patients who had failed interferon therapy. The median expanded disability status scale (EDSS) score prior to transplantation of patients was between 6.5 and 7. Data indicates that both the course of MS and the severity of the disabilities at the time of treatment influence the outcome of transplantation. Progression of disabilities and continuing relapses following transplantation are greater in patients with advanced pretransplant disabilities (54–56,60). In aggregate, evidence of ongoing MS after transplantation was three times more frequent for patients with a pre-transplant EDSS score greater than 6.5 when compared with those patients with an EDSS less than 6.0.

Intervention with transplantation has often been delayed until the patient’s quality of life is felt to justify the risks of transplant related mortality, yet 3 of 47 patients died within two years of the transplant from progressive MS in the two studies (54,55) that enrolled patients with the most advanced disabilities. By comparison, only one patient died of transplant related complications. Treating patients with less advanced disabilities appears to be a more rationale way to optimize the effectiveness of these treatments.

The clinical and radiological data support the idea that patients with less accumulated disability have a greater chance of responding to treatments directed at modifying the immune system, given that inflammatory events are the prominent mechanism of disease at this time in the course of MS. Nevertheless, the significant risk of morbidity and mortality associated with high dose cytotoxic therapy, treating young, less disabled patients may appear too costly to the patients and their physicians. On the other hand, waiting to intervene until patients have progressed to have significant disabilities may be more acceptable, but such patients are more likely to have ongoing neurodegenerative processes and will be unlikely to respond to a treatment directed at mitigating the detrimental effects of CNS inflammation (92).

**FUTURE DIRECTIONS—BEYOND CYTOTOXIC IMMUNOSUPPRESSION**

Currently, immune suppression in MS is achieved by the use of cytotoxic agents. Cellular immune responses provide an alternate approach to immune ablation. Donor lymphocytes in stem cell grafts can attack and destroy recipient lymphocytes based upon differences in minor histocompatibility gene loci between the donor and recipient. Nonmyeloablative doses of cytotoxic drugs prevent rejection of the allogeneic graft (93,94). This graft-versus-host lymphocyte reaction is already being used in allogeneic reduced-intensity conditioning (RIC) transplants for the treatment of low-grade lymphoproliferative diseases (95,96). Regimen related toxicity is low but incomplete control of the allograft reaction can lead to acute or chronic GVHD, a syndrome with significant morbidity and mortality (93). A number of patients who have undergone RIC transplants for cancer have had improvement in coexisting autoimmune diseases (94,97,98). Trials of allogeneic RIC transplantation in autoimmune diseases are being proposed (99).
Mesenchymal stem cells (MSC), bone marrow derived cells that repopulate the stromal elements of the marrow, can constrain immune reactions and have even been shown to suppress life-threatening GVHD (100). The suppression is achieved through nonlymphocytotoxic mechanisms (101,102). MSC are easily harvested by a bedside procedure and can be greatly expanded in vitro. This immunosuppressive effect has attracted the attention of researchers and could one day be harnessed to treat autoimmunity.

FUTURE DIRECTIONS—BEYOND REPAIR OF THE IMMUNE SYSTEM

Stem cells, specialized cells responsible for development and regeneration, exist in many organs in the body. While controversial, there is mounting evidence that primitive stem cells from one organ may be able to repair other damaged organs. Stem cell graft derived cells have been found in the many different organs in the body of bone marrow transplant recipients (103), including the brain (104,105). They can differentiate into oligodendrocytes and neurons in vitro and in vivo. MSC can migrate to sites of myelin damage in rodent models, differentiate into functional oligodendrocytes and aid functional recovery (106,107). Reparative signals from a damaged brain can drive the fate of stem cells in the body (108). Functional recovery occurred in rats, given a stem cell transplant, following stroke (109). Repair of the damage caused by MS may become possible as the regulatory signals modulating stem cell fate decisions are elucidated.

CONCLUSION

Overall in MS, the experience using cytotoxic drugs in conventional or at high doses in combination with HSCT support the hypothesis that greater immune suppression is associated with better disease control. More intense regimens are associated with added treatment related morbidity and mortality. Is it better to use a less aggressive preparative regimen and reset the autoimmunity to an earlier stage in the MS course or is it better to accept the toxicity required to eliminate the autoreactive process and provide definitive disease control? What is the optimal time in a patient’s illness to intervene with this promising yet toxic treatment? Perhaps the answer is different for patients with each of the various distinct patterns of MS. Future studies will be directed at balancing the intensity of the treatment with the treatment outcome.

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Combination Therapy in Multiple Sclerosis

Mark J. Tullman and Fred D. Lublin
Corinne Goldsmith Dickinson Center for Multiple Sclerosis, Mount Sinai School of Medicine, New York, New York, U.S.A.

INTRODUCTION

The treatment of multiple sclerosis (MS) has been revolutionized over the past decade. Just 12 years ago, MS was not considered a treatable neurologic illness and our therapeutic armamentarium consisted largely of symptomatic therapies and corticosteroids to treat acute exacerbations. Currently, there are five drugs [the three beta interferons (IFNs) (Avonex\textsuperscript{R}, Betaseron\textsuperscript{R}, and Rebif\textsuperscript{R}), GA (Copaxone\textsuperscript{R}), and mitoxantrone (Novantrone\textsuperscript{R})] representing three different classes of agents, approved by the Food and Drug Administration (FDA) and available in the United States, that alter the course of MS (1–5). However, these therapies provide a rather modest benefit and none results in complete disease control. Consequently, many patients continue to have exacerbations and accumulate disability and demyelinating lesions in the central nervous system (CNS).

To improve upon the existing MS therapies, the efficacy and safety of novel treatment approaches need to be established. However, with the advent of partially effective therapies, we are now confronted with new challenges in developing better disease-modifying agents and treatment strategies. Because roughly 40% of MS relapses result in persistent neurological deficit, there are major ethical concerns about withholding proven therapies to conduct placebo-controlled trials in relapsing–remitting (RR) MS (6). Equivalence trials generally do not meet FDA regulatory requirements for drug approval in the United States and superiority trials require large sample sizes, which make recruitment difficult and may drastically limit the number of trials that can be performed (7). One strategy to improve upon the current state of MS treatment is to combine therapies. A similar approach has been successful in treating other autoimmune and infectious diseases such as rheumatoid arthritis and human immunodeficiency virus (8,9).

SELECTING AGENTS FOR COMBINATION THERAPY

Once widely believed to be primarily an inflammatory demyelinating disease of the CNS, it is now apparent that MS is also a degenerative disease resulting in axonal injury
and neuronal loss (10,11). As such, when selecting agents for combination therapy, careful consideration should be given to the pathophysiology of MS as well as the mechanism of action, potential therapeutic effects, and adverse effects of each monotherapy. Even so, immunomodulatory agents do not always perform as expected when used alone and it will likely be more difficult to predict their effects when used in combination (12). A particularly appealing strategy is to combine agents with very different mechanisms of action, such as an anti-inflammatory agent with a neuroprotective one to attack both the inflammatory and degenerative aspects of the illness.

Based on desperation and anecdotal reports, immunosuppressive and cytotoxic drugs are often combined with immunomodulatory agents in patients with a suboptimal response to the proven disease-modifying therapies. However, many of these drugs have never been conclusively demonstrated to be beneficial in altering the course of MS when used alone, and some have potential serious side effects (13–15). Some neurologist combine mitoxantrone with interferon (IFN)-β or GA, but it is unknown whether such a combination is useful or even necessary. Several other combinations have been reported to be effective and well tolerated in small, open-label MS trials (16,17). However, anecdotes and uncontrolled studies are merely observations and in an unpredictable and variable illness like MS, any perceived treatment effect needs to be confirmed in a rigorously controlled trial.

Ultimately, the success (or failure) of combination therapy will depend on the mechanism of action of the agents being used and their collective safety and tolerability profile. In November 2004, Natalizumab (Tysabri®), a humanized monoclonal antibody, was approved by the FDA for relapsing forms of MS based on the preliminary results of two studies (18). One of these trials compared natalizumab with a placebo in mostly treatment-naive RRMS patients and the other examined the combination of natalizumab and IFNβ-1a (the Avonex form) versus IFNβ-1a alone in RRMS patients who had experienced at least one relapse in the previous 12 months despite treatment with IFNβ-1a. Natalizumab, a selective adhesion molecule blocker, which limits the trafficking of T-lymphocytes into the CNS, appeared to be a significant advance in MS therapeutics. Unfortunately, just three months after it was approved, the drug was withdrawn from the U.S. market after two patients in the combination study who had received over two years of therapy with IFNβ-1a and natalizumab developed progressive multifocal leukoencephalopathy (PML) (19). One of these cases was fatal. Natalizumab has been studied in other autoimmune diseases and a subsequent safety analysis of the approximately 3000 patients who participated in the MS, Crohn’s disease, and rheumatoid arthritis–natalizumab trials revealed a third case of PML. In December 2003, a patient with Crohn’s disease, who had received eight doses of natalizumab over 18 months and prior immunosuppressive agents died from what was thought to be a malignant astrocytoma (20). Following the reports of PML in MS patients, the histopathology of a brain biopsy specimen from the Crohn’s patient was re-reviewed and the diagnosis was changed to PML. Thus far, there are no known cases of PML occurring in patients treated with natalizumab monotherapy. Nonetheless, it remains unclear if the association between PML and natalizumab is related to natalizumab alone or its combined use with other immunosuppressive or immunomodulatory agents, and the future prospects of this seemingly once-promising MS therapy have been greatly diminished. Natalizumab has now been added to the growing list of agents that were reported to be safe and effective in animal models of MS or early phase MS clinical studies that were subsequently disproved or associated with unexpected adverse effects in larger, double-blinded, placebo-controlled trials (21–23).
The problems that arose with the use of natalizumab raise cautionary flags about both single and combined use of immunomodulatory agents. However, also to be gleaned from this event is the potential for using combination therapy to allow for lower dosing of individual agents and potentially fewer dose-related adverse effects.

**IFNβ AND GA**

IFNβ and GA are well suited for testing their combinatorial potential, as their mechanisms of action differ and could be complimentary. Furthermore, when used alone, they are partially effective, safe, and generally well tolerated (1–4).

The therapeutic effects of IFN may be due to its antiproliferative action; downregulation of costimulatory molecules; decrease of proinflammatory cytokines; and/or through its effects on matrix metalloproteinases and adhesion molecules, which reduce the permeability of the blood–brain barrier and limit trafficking of T-lymphocytes into CNS (24). The beneficial effects of GA may result from reactive Th2 cells that cross the blood–brain barrier and increase the secretion of suppressor type cytokines and downregulate inflammatory activity within the CNS—a process known as bystander suppression (24).

Another interesting aspect of using GA in combination with IFN relates to the growing body of evidence suggesting that in addition to its immunomodulatory actions, GA may also have a neuroprotective effect. A recent study found that GA may favorably affect the development of T1-hypointense lesions on brain magnetic resonance imaging (MRI), suggesting a reduction in the development of underlying axonal damage in evolving MS lesions (25). Additional evidence that supports the concept of GA as a neuroprotective agent is the report of secretion of brain-derived growth factor by GA-specific T-cell lines (26). However, there is potential concern that IFN could negate the beneficial effects of GA. If IFN shores up the blood–brain barrier, it might prevent GA-induced Th2 cells from entering the CNS. Furthermore, the antiproliferative effects of IFN could inhibit the generation of GA-reactive Th2 cells. Studies in experimental allergic encephalomyelitis, an animal model of MS, utilizing oral IFN alpha and either subcutaneous or oral GA, suggested that the combination is less effective than either therapy alone (27). However, an in vitro study of the effects of combining IFNβ-1b and GA suggests an additive effect of the combination (28). A multicenter, open-label trial subsequently provided evidence that the combination of GA and IFNβ-1a (the Avonex form) is safe for use in MS patients (29). In this study, 31 patients with RRMS with an expanded disability status scale (EDSS) score between 0 and 5.5, who had been taking IFNβ-1a injections weekly for at least six months, had GA 20 mg subcutaneously (s.c.) daily added to their regimen after a three-month run-in period. MRIs were obtained monthly for the duration of the study (for three months prior to and then for six months after the initiation of GA). The primary objective of this study was to determine whether the combination of the two agents was safe, as determined by the number of gadolinium (Gd)-enhancing lesions on MRI scans during the six months of combined treatment versus the run-in period of three months of IFN monotherapy. Secondary outcome measures included change in EDSS, the MS functional composite (MSFC), and relapse rate. Twenty-six subjects completed six months of combination therapy. There was no increase in Gd-enhancing lesions during the six months of combination therapy. In fact, the mean number of enhancing
lesions decreased from 0.88 on the baseline scans to 0.44 during the six months of combined therapy—a 47% reduction. However, there was a statistically significant decline in the mean number of Gd-enhancing lesions during the three-month run-in period when patients received IFN therapy alone, illustrating some of the difficulties of interpreting open-label data (Fig. 1).

There was no major change in relapse rate during the study; two patients experienced relapses during the run-in phase and three patients had relapses over the course of six months of combination therapy. As expected, there was no significant change in EDSS during this short study. The injections were well tolerated and there was no significant change in laboratory measures.

At the end of six months of combined treatment, the study was extended an additional six months with MRI being performed at months 9 and 12 (30). Sixteen of the 17 subjects who entered the extension phase of the study completed 12 months of combination therapy. Thirty-two percent of patients had Gd-enhancing lesions during the first six months of therapy while only 12% of patients had enhancing lesions during the six-month extension phase, a time during which the MRI effects of Copaxone would be expected to become evident (31). However, these results should be interpreted with caution, as MRI scans were performed less frequently during the extension phase than in the original six-month study. Although there was no change in the MSFC, a significant improvement in walking speed was apparent over the entire 12 months of the study. There were no exacerbations or new safety concerns during the final six months of combination therapy.

The potential therapeutic benefit of the combined use of IFN and GA was further supported by an immunologic study in a subgroup of patients enrolled in this trial (32). In this study, the proliferative response of T-cells to GA in five patients treated with IFN and GA was compared with a control group consisting of MS patients treated with GA alone. There was no evidence that IFN inhibited proliferation of GA-reactive T-cells and there was a similar Th1 to Th2 shift in both groups, indicating that IFN did not interfere with the immunomodulatory effects of GA.

In 2003, the National Institutes of Health/National Institute of Neurological Disorders and Stroke funded a phase III study of combination therapy in MS based

![Figure 1](image-url)  
**Figure 1** Mean number of gadolinium-enhancing lesions before and during combination therapy with IFNβ-1a and glatiramer acetate. *Abbreviation:* IFNβ, interferon beta.
on these preliminary results. This double-blind, randomized, three-arm trial will involve 1000 subjects with RRMS from 70 centers across North America and compare the efficacy of the combined use of IFNβ-1a (the Avonex form) and GA to the efficacy of either agent when used alone in treatment naive RRMS patients. Subjects will be randomized to one of three groups in a 1:1:2 ratio: 25% of subjects will receive IFNβ-1a once weekly and a daily s.c. placebo; 25% will get GA daily and a weekly intramuscular (i.m.) placebo; and 50% will receive both active drugs. The primary outcome measure of this three-year study is the reduction in annualized relapse rate. Secondary objectives include confirmed EDSS progression, change in MSFC, and MRI measures of disease. In addition to assessing the primary question of the effectiveness of combined therapy, by utilizing a three-arm design, this trial is also powered to provide head-to-head data comparing the efficacy of IFNβ-1a to GA and will be the first double-blind, placebo-controlled trial comparing the efficacy of two proven immunomodulatory agents in MS. Enrollment in this trial is currently underway, and results should be available in 2009.

**IFN, METHYLPREDNISOLONE, AND METHOTREXATE**

Corticosteroids have anti-inflammatory and immunosuppressive properties and have been used to treat acute MS exacerbations for more than 30 years (33). While periodic pulses of intravenous (IV) methylprednisolone are not effective in preventing disability in patients with progressive MS, a phase II trial concluded that they do have an effect on disability, brain atrophy, and T1-hypointense lesions in patients with RRMS (34,35).

Methotrexate impairs DNA and RNA synthesis by inhibiting dihydrofolate reductase and has potent immunosuppressive and anti-inflammatory activity. It was studied in patients with progressive MS in a randomized, double-blind, placebo-controlled trial. In this trial, 60 patients with progressive forms of MS with EDSS scores of 3.0 to 6.5 were randomized to receive methotrexate 7.5 mg or placebo orally every week for two years (36). The primary endpoint was the rate of sustained disability progression as determined by a composite of four clinical outcome measures. After two years, there was a significant treatment effect: 83% of patients in the placebo-treated group had sustained disability progression compared with 52% in the methotrexate-treated group. However, when the components of the composite were analyzed individually, there was a significant effect on one measure of upper extremity function, but not on ambulation or EDSS. Fifty-six of the 60 patients in this study had at least one annual MRI scan with Gd. No significant difference existed between the two groups in change from baseline in T2 total lesion area at one and two years. Gd-enhancing lesions were uncommon in both groups. Methotrexate was well tolerated in this study. However, major toxicities are associated with long-term use of low doses of methotrexate, including pulmonary fibrosis, hepatoxicity, and bone-marrow suppression (37).

Methotrexate was studied in combination with IFN in an open-label fashion (38). To be eligible for this study, patients had to meet clinical and MRI criteria. The clinical criteria included: (i) relapsing MS, (ii) EDSS 0.0 to 6.0, (iii) treatment with IFNβ-1a (the Avonex form) for a minimum of one year, and (iv) at least one relapse after three months of IFN therapy. Patients who fulfilled these criteria were eligible to participate in this study if they had at least two Gd-enhancing lesions on three monthly baseline-screening brain MRI scans. In this study, 15 patients received
oral methotrexate 20 mg weekly in addition to IFN for six months. The primary outcome was safety and tolerability as determined primarily by adverse events, hematologic studies, and serum chemistries. A number of clinical and MRI measures served as secondary endpoints. The combination of IFN and methotrexate was safe and well tolerated. Nausea occurred in most patients for up to 24 hours following methotrexate therapy. Compared to the baseline-screening period when patients were treated with IFN monotherapy, there was a significant 44% reduction in the mean number of Gd-enhancing lesions during the final three months of combination therapy. However, these results should be interpreted with caution. In contrast to the IFN and GA combination open-label study where there was no MRI inclusion criterion, the design of this study was such that combination therapy was initiated at a time when patients had active MRI scans. The observed treatment effect of combination therapy may reflect nothing more than regression to the mean (i.e., spontaneous improvement). There was a nonsignificant 63% reduction in the mean number of relapses during the six months of IFN and methotrexate therapy compared to the six months before combination therapy was initiated. There was no significant change in the mean EDSS of MSFC during combination therapy.

A large, multicenter, blind, placebo-controlled study to determine the safety and efficacy of combining IFNβ-1a (the Avonex form) with IV methylprednisolone, methotrexate, or both is currently underway (39). In this trial, approximately 900 patients with RRMS, an EDSS of 0.0 to 5.0, and at least one Gd-enhancing lesion or relapse in the previous year while on IFNβ-1a will be randomized to receive additional treatment with an oral placebo weekly, oral methotrexate 20 mg weekly, oral placebo weekly and a three-day course of IV methylprednisolone bimonthly, or both active drugs for two years. For methotrexate, this a double-blind, placebo-controlled study with a primary outcome measure of relapse rate. For methylprednisolone, the study is observer blind, and the primary outcome is a measure of brain atrophy. Other efficacy measures for both agents include change in the MSFC, EDSS progression, and various MRI parameters, including T1-hypointense and Gd-enhancing lesions. Results of this trial will not likely be available before 2008.

MITOXANTRONE AND METHYLPREDNISOLONES

In 2001, mitoxantrone, an anthracendione with immunosuppressive and immunomodulatory properties, was approved by the FDA for the treatment of secondary progressive and worsening RRMS based largely on the results of a multicenter, randomized, blind, placebo-controlled, two-year trial involving 194 MS patients (4). However, an earlier multicenter, randomized, single-blind, controlled study demonstrated the benefit of mitoxantrone combined with methylprednisolone in patients with severe MS (40). Patients were initially considered for this study if they had RRMS and at least two relapses with incomplete recovery or secondary progressive MS and an increase of at least two EDSS points during the previous 12 months. These patients were treated with IV methylprednisolone 1 g every month and underwent monthly screening MRI scans for three months. To be included in this trial, patients had to have at least one Gd-enhancing lesion on the screening MRIs. Forty-two patients (76% of whom had RRMS) were subsequently randomized to treatment with methylprednisolone 1 g and mitoxantrone 20 mg, both as an IV infusion each month, or IV methylprednisolone 1 g/month alone for six months. In four out of six months, the combination of mitoxantrone and methylprednisolone was
superior to methylprednisolone in the proportion of patients without new enhancing lesions on monthly MRIs—the primary outcome measure of this study (Fig. 2). The beneficial effect was seen following two months of treatment and after six months of therapy, 91% of patients receiving mitoxantrone and methylprednisolone had no new enhancing lesions compared to just 31% of those receiving only methylprednisolone (P < 0.001). There were also significant effects of combination therapy on relapse rate, EDSS, and other MRI measures of disease. It must be pointed out that MRI outcomes were assessed by blinded observers, but clinical outcomes were determined in an open-label fashion. The design of this study is such that one cannot determine if it is necessary to use both mitoxantrone and methylprednisolone. Nonetheless, the combination of the two drugs appears to be safe and effective in patients with highly active disease.

When used alone, the major toxicities of mitoxantrone are bone marrow suppression and cardiotoxicity, which is usually dose related. For women, particularly those older than 35, infertility is a concern, and there are also rare reports of secondary leukemias in MS patients treated with mitoxantrone (41). The combination of monthly mitoxantrone and methylprednisolone was generally well tolerated and there was no evidence of cardiotoxicity. Adverse events were similar to what would be expected from mitoxantrone monotherapy.

CONCLUSIONS

Given the limited efficacy of the MS immunomodulatory agents and the challenges associated with performing placebo-controlled, equivalence, and superiority trials, the logic of combining therapies in MS has considerable appeal. However, selecting
agents for combination requires careful consideration because immunomodulatory agents do not always have the desired effects, the immunomodulating activity of one drug could potentially interfere with the therapeutic effect of another, and certain combinations may be associated with unforeseen adverse events. Hopefully, the results of ongoing and future studies will provide valuable insight into the most effective use of new and existing MS therapies.

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Regeneration Strategies for Multiple Sclerosis

Arthur E. Warrington and Moses Rodriguez
Departments of Neurology and Immunology, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

INTRODUCTION

Demyelination is the pathologic hallmark of the multiple sclerosis (MS) lesion and has long been believed to be the underlying cause of neurologic deficits. However, demyelination is accompanied by varying degrees of inflammation, oligodendrocyte death, axonal loss, complement activation, antibody deposition, and gliosis (1–4). With the development of magnetic resonance imaging (MRI) patients who present with minimal or no neurologic deficits are routinely identified with extensive white matter lesions. Pathologic examination of central nervous system (CNS) tissue at biopsy or autopsy has confirmed that lesions visible by MRI were demyelinated and often involved substantial areas of the CNS that should have resulted in neurologic deficits (5). It is now clear that loss of axons is the ultimate cause of permanent disability rather than demyelination.

Animal models also support the premise that demyelination is necessary, but not sufficient for the development of permanent neurologic deficits. Demyelination likely predisposes axons to permanent injury via a second, likely immune mediated, assault. In a virus mediated mouse model of MS, deleting the major histocompatibility (MHC) class I antigen presenting arm of the immune response results in mice in which striking spinal cord demyelination exists without neurologic deficits (6). Axon function is largely preserved, likely due to an increase and redistribution of sodium channels along the demyelinated axons. A role of MHC class I in the induction of neurologic deficits implicates CD8+ T-cells as the pathologic effector cells. Perforin and Fas ligand, a member of the tumor necrosis factor superfamily, are two molecules synthesized by CD8+ T-cells that mediate membrane cytolysis. Perforin-deficient mice infected with a demyelinating virus develop persistent and chronic demyelination, but present with only minimal neurologic deficits (7). These studies support the hypothesis that CD8+ T-cells are the immune component that directly assaults demyelinated axons. Protecting axons from external injury and providing neurotrophic support to axons may be the primary function of the mature myelinating oligodendrocyte. If this is true, then any strategy that promotes remyelination within a critical time period will ultimately be neuroprotective. How long a human axon can remain demyelinated and remain viable is unknown.
REMYELINATION AS A NORMAL REPARATIVE RESPONSE

Spontaneous remyelination of demyelinated MS lesions does occur (8–10). Remyelinated axons are visible pathologically as abnormally thin sheaths, usually at the periphery of the lesion. Remyelination in acute MS lesions can often be substantial (11). As many as 70% of MS lesions contain some degree of remyelination (12) and full repair may be possible in the early stages of disease. Periods of remission are likely associated with significant CNS remyelination. In contrast, remyelination in chronic lesions is extremely limited. Therefore, interventions early in disease to stimulate reparative cells or to remove inhibitory factors preventing myelin repair may be key to a therapeutic strategy (13). Why remyelination so frequently fails in MS remains unknown. A number of reasons have been proposed for the failure of complete remyelination in MS and likely vary in their contribution to the disease between individuals. The depletion of cells capable of remyelination, the depletion of factors that sustain the growth and differentiation of myelinating cells, and an environment inhibitory to the remyelination process may all underlie the failure of remyelination (14).

The strong remyelination response that is observed in acute MS lesions mirrors the robust remyelination observed in animal models following various experimental demyelinating strategies. Experimental demyelination can be induced by toxins such as cuprizone (15), ethidium bromide (16), or lysolecithin (17). Demyelination can also be induced by autoimmune mechanisms (experimental autoimmune encephalomyelitis (EAE) (18), or by virus infection such as coronavirus (murine hepatitis virus (MHV) (19) or picornavirus, theiler’s murine encephalomyelitis virus (TMEV) (20,21). Spontaneous remyelination has been demonstrated in each of these models. Demyelination resulting from the injection of the detergent lysolecithin is rapidly and completely remyelinated (22,23). Remyelination restores axonal conduction, which in turn leads to the recovery of motor function (24–26). MHV-induced demyelination in mice is accompanied by ataxia and paralysis, but following virus clearance complete remyelination reverses functional deficits (27,28). In contrast, spontaneous remyelination is limited following TMEV infection, where there is persistence of an immune response directed against chronic virus antigen. These model systems suggest that remyelination is a normal reparative response following injury. The most obvious difference between human MS and these models of acute demyelination is a persistent activation of the immune system.

PRESENT TREATMENTS FOR MS TARGET INFLAMMATION, NOT REPAIR

A general consensus that MS is primarily immune mediated has logically led to investment in immunomodulatory therapies. Current treatments for MS focus on controlling the early inflammation based, MRI visible, phase of the disease. This approach assumes that controlling inflammation will limit demyelination and permanent neurologic deficits. Many conventional immunomodulatory treatments and general immunosuppressants have been tested for efficacy in MS (29), but none had sufficiently positive effects to warrant approval. Current treatments have been approved for clinical use based on a decrease of relapse rate in short-term trials and of gadolinium-enhancing MRI lesions, which is a surrogate for reduced inflammation. In practice, current treatments for MS have no effect on permanent
and accumulating deficits. Demyelination and inflammation are likely independent contributors to the development of lesions. The major therapeutic focus may need to shift to encouraging early and rapid remyelination, the ultimate goal of which is to prevent axonal dysfunction, injury and loss, rather than limiting inflammation.

The limited therapeutic benefit of current immunomodulatory therapies may be due in part to the pathogenetic and clinical heterogeneity of MS (12). Understanding this heterogeneity may allow therapeutics to be targeted to subgroups of MS patients who are most likely to respond. For example, in active MS lesions with pronounced immunoglobulin, complement deposition and only moderate loss of oligodendrocytes, removal of auto-reactive antibodies would likely be of benefit. Therapeutic plasma exchange, which removes immunoglobulin and complement along with many other blood components, resulted in significant clinical improvement in a subset of patients who experienced corticosteroid unresponsive severe neurologic deficits after attacks of inflammatory demyelinating disease (30,31).

Of particular importance, immunosuppressive therapies may not be efficacious in MS because inflammation may be required for effective CNS repair (32,33). CNS inflammation likely consists of beneficial elements, the purpose of which is to facilitate tissue repair as well as elements contributing to the injury. Analysis of active MS lesions (34) and spinal cord lesions in mice chronically infected with TMEV (35) documents that remyelination proceeds even in the presence of inflammation. Future treatments for MS need to selectively alter the inflammatory balance, not merely reduce all aspects of inflammation.

INFLAMMATION HINDERS AS WELL AS FACILITATES CNS REPAIR

The CNS is often considered a site of immune privilege based on the physical separation of CNS tissue from peripheral immune function by the blood–brain barrier. In reality, the isolation of the CNS is often imperfect. There are many examples in human disease and animal models of disease in which the cellular and humoral branches of the immune system interact with the CNS. Autoimmune responses directed against the CNS have generally been considered pathogenic and there are well documented conditions in which this is the case. However, the traditional concept that all inflammation is necessarily detrimental to CNS repair and that an immune response directed against CNS antigens is necessarily pathogenic have been challenged. It is clear that manipulating cellular or humoral components of the immune system can promote CNS repair or protect the CNS from pathologic damage (36–41).

Interfering with the function of T-lymphocytes in animal models can improve CNS repair. The presence of either CD4+ or CD8+ T-cells can restrict the level of remyelination. Immunosuppression with cyclophosphamide or lymphocyte depleting antibodies directed at CD4 or CD8 T-cells promotes fivefold to sevenfold more remyelination in chronically TMEV infected SJL mice (42). PLJ mice, which are genetically deficient in CD4+ T-cells, remyelinate and recover from virus induced neurologic deficit in contrast to normal mice (43). Mice with a genetic deletion of beta 2-microglobulin, which are unable to make MHC class I restricted CD8+ T-cells, also spontaneously repair with minimal neurological deficits (6,44). These studies indicate that factors associated with immune T-cells can impair remyelination. It needs to be stressed that in these model systems spinal cord remyelination occurs in mice depleted of selected T-cells despite the persistence of virus in the CNS.
However, there are other examples where immune cells and their effector molecules appear to facilitate myelin repair (45–47). When a focal demyelinated lesion is induced by lysolecithin in the spinal cord of B6 wild type mice remyelination proceeds rapidly and completely. In contrast, following a similar lesion in B6 Rag-1 mice, which produce no mature T- or B-cells, remyelination is substantially impaired (48). Focal spinal cord demyelination was induced in mice genetically deficient for or directly depleted of CD4\(^+\) T-cells, important in MHC class II restricted immune responses or animals genetically deficient for CD8\(^+\) T-cells, important in MHC class I restricted immune responses. The absence of either subset of T-cells greatly reduced the level of spontaneous remyelination. Depleting macrophages also impairs remyelination, suggesting that these cells are also important for the support of remyelination (47).

A role for T-lymphocytes in protecting axons following CNS injury has also been proposed (49,50). Systemic injection of myelin basic protein reactive T-cells following spinal cord injury resulted in the enhanced accumulation of T-cells, B-cells, and macrophages at the site of injury (51). This T-cell directed response drives the increased expression of several neurotrophic factors by local macrophages and astrocytes that may aid in promoting neuronal survival. T-cells may play a similar role in promoting remyelination. The level of several growth factors expressed by T-cells with a demonstrated effect on the oligodendrocyte lineage are increased during periods of remyelination (52,53).

This cell based response induced following CNS injury is proposed to be a normal aspect of the immune repertoire, but the level of response is usually insufficient to facilitate significant CNS repair. The phenomenon of preconditioning—an initial traumatic injury to the CNS that facilitates an increase in systemic factors that improves CNS repair upon a subsequent injury at a distant site (54)—provides direct evidence of an innate immune based reparative program. More surviving neurons were measured in the optic nerve of animals which received an earlier lesion to the spinal cord than those that underwent sham surgery. The transfer of CNS antigen activated splenocytes from CNS lesioned animals substituted for the neuroprotective effect of a prior spinal cord lesion. The fact that the spleen contains antibody producing B-cells leaves open the possibility that the reported protective immune response may be either cellular or humoral based. Activating the appropriate autoreactive T-cells to drive CNS repair will likely be a complex task (55).

**GROWTH FACTORS FOR MS LESION REPAIR AND REGENERATION**

The treatment of MS with soluble growth factors or cytokines to promote remyelination assumes that the injured CNS has cells capable of synthesizing myelin, but that the environment does not support myelinosogenesis. Extensive animal studies have defined crucial factors required for the survival, proliferation and differentiation of cells of the oligodendrocyte lineage. Factors with demonstrated effects on oligodendrocytes include platelet derived growth factor (PDGF-\(\alpha\)) (56,57), fibroblast growth factor 2 (FGF-2) (58–60), neuregulin-1 (61), chemokine (C-X-C motif) ligand 1 (CXCL1) (62), insulin like growth factor I (63,64) thyroid hormone (65,66), neurotrophin-3 (67,68), ciliary neurotrophic factor (69), and leukemia inhibitor factor (70). Oligodendrocyte progenitors can be maintained in a proliferative state by the combination of PDGF-\(\alpha\) and FGF2 (71,72), both factors of which are expressed in CNS lesions (73,74). In theory, administering these factors to the appropriate location
in vivo could expand the available pool of myelinating cells. Most studies defining the factors involved with remyelination have been carried out in acute focal demyelinated lesion models that present with a transient inflammatory cell infiltration to the lesion. Whether trophic factors and cytokines will be effective in the presence of chronic inflammation and therefore translate to the treatment of MS is largely untested.

Growth factor therapy may appear attractive, but there are several unresolved issues surrounding this type of therapy. There is an evolving appreciation that myelogenesis requires combinations of multiple factors available to cells in a correct sequence and timing (52,75). What growth factors to use for MS and when and how to administer them is difficult to determine. The pathology of the MS lesion and the stage of differentiation of surviving myelinating cells will need to be determined before making this decision. Administration of the incorrect cytokine may interfere with remyelination (76) or trigger apoptosis in differentiated oligodendrocytes (77). If an individual’s demyelinated lesions are lacking a critical level of oligodendrocyte progenitors, then factors that recruit new oligodendrocyte progenitors to the lesion and that expand existing myelinating cells may be beneficial to repair. However, administering factors that drive oligodendrocyte progenitors prematurely toward differentiation may limit the extent of remyelination. There are also clear differences between human oligodendrocyte lineage cells and their better characterized mouse and rat counterparts. For example, human oligodendrocyte progenitors do not respond to mitogens known to trigger proliferation in rodent cells (78,79). The above issues combined with the generally demonstrated pleiotrophic effects of most cytokines and the difficulties of controlled, targeted, and sustained factor delivery to the CNS, limit the use of growth factor therapy for MS at the present.

CELL TRANSPLANTATION FOR MS LESION REPAIR AND REGENERATION

Despite ineffective remyelination in MS, abundant numbers of oligodendrocytes and their progenitors are present even in chronic MS lesions (80–82) emphasizing that environmental factors in addition to the absolute number of myelinating cells contribute to the lack of remyelination. In animal models of CNS demyelination, remyelination is accomplished by recruiting endogenous myelinating cells from adjacent intact tissue (83) or by the migration of undifferentiated neural precursor cells from germinial zones to the lesion site where they proliferate and differentiate into myelinating cells (84). Why these surviving cells fail to respond to tissue injury and demyelination in MS may be due to a number of reasons. Chronic virus infection may render myelinating cells incapable of synthesizing the metabolically intensive myelin membrane. Inflammatory factors that interfere with remyelination may dominate those that are reparative. Myelinating cells within the CNS may be depleted below a critical threshold and myelinating cells from adjacent areas are unable to effectively migrate to the lesion. The progressive loss of axons would result in fewer substrates to remyelinate.

The transplantation of remyelination competent cells is a clinically relevant approach to promote regeneration in MS. The principle underlying cell transplantation strategies is that the damaged CNS has exhausted its cells capable of remyelination or that the surviving oligodendrocytes are incapable of recognizing bare axons and elaborating new myelin. Numerous experimental studies have demonstrated that oligodendrocytes or their progenitors survive, proliferate, migrate, and
myelinate when transplanted directly into dysmyelinated mutant animals or experimentally demyelinated lesions (85–87). Remyelination by transplanted glial cells can restore spinal cord conduction (88) and neurologic function (89).

Unresolved issues in transplantation include the choice of cell for transplantation and the method of delivery. In a multifocal disease such as MS, it is impractical to stereotactically implant cells directly into every demyelinated lesion. The only viable approach is a systemic delivery of cells that then find their way into the CNS and specific areas of damage. Glial cells implanted into the CNS at a distance from a demyelinated lesion can migrate toward the area of damage (90) suggesting that soluble factors released from the area of injury can guide exogenous as well as endogenous reparative cells (84). The observation that the intact adult CNS is an unsupportive environment in which to place oligodendrocyte progenitors complicates the potential use of glial transplantation therapy (91).

Embryonic stem (ES) cells and multipotential neural stem cells are, at present, the most promising sources of remyelination competent cells. Each can be expanded almost without limit and differentiated in vitro (92). Neural stem cells have been isolated from diverse regions of the developing and adult rodent and human CNS (93). Once transplanted into the CNS, neural stem cells can adapt to the region of engraftment by differentiating into the appropriate neuronal and glial subpopulations (94,95). Transplanted ES cells differentiated into oligodendrocytes when transplanted into the injured rat spinal cord (96,97). Glial precursors derived from embryonic stem cells or neural precursors myelinate following transplantation into the CNS (98,99). Intraspinal delivery of neural stem cells into the MHV induced model of demyelination resulted in extensive migration of transplanted cells, remyelination, axonal sparing, and behavioral improvement (100). In models of demyelination where the blood–brain barrier is open, such as EAE, intraventricularly implanted neural precursors entered the CNS and differentiated into myelinating oligodendrocytes (101).

Recent studies of transplanted cells into models of demyelination suggest that these cells increase the level of remyelination by inducing repair by endogenous myelinating cells, rather than directly proliferating and myelinating themselves (100,101). Neural stem cells infiltrating the CNS may act as localized cytokine and growth factor factories (102) activating the remaining oligodendrocyte progenitors. If this is true then a small number of transplanted cells correctly targeted may have widespread effects on lesion repair. Transplanted cells may also be viewed as antigens themselves. Their presence within the area of injury may stimulate immune cells to increase cytokine synthesis. The transplantation of immune cells themselves into the injured spinal cord can activate endogenous stem cells (103). As with growth factor based repair strategies most transplantation based remyelination studies have been carried out in dysmyelinating mutant animals or acutely demyelinated lesions (104–106). Limited data exists on the efficacy of cell transplantation to repair chronic immune mediated demyelinating disease. Inflammation may be essential to remove damaged tissue, especially myelin, which contains molecules that inhibit cell migration and axonal regrowth (107) or to guide reparative cells. Activated microglial cells concentrated at sites of CNS injury release soluble factors that direct neural stem cell migration and differentiation (108).

The limitation of embryonic and neural stem cells is that these cells need to be obtained from embryonic tissue or CNS biopsy material. Bone marrow stromal cells are an attractive alternative for autologous cell transplantation based repair. Bone marrow transplantation is used routinely to treat a variety of human disorders with
no evidence of abnormal cell proliferation. The risk of uncontrolled in vivo cell proliferation remains with all cells that can be maintained in a proliferative state in culture. Bone marrow extracted from the long bones can be separated based on surface antigen expression, expanded in culture, driven toward various fates and reintroduced into patients. Rodent bone marrow cells can differentiate into myelin forming cells when transplanted into a focal demyelinated lesion (109–112). However, not all studies of hematopoietic stem cell transplants into demyelinated models have reported myelin formation (113,114). Human bone marrow cells have not been rigorously tested for their in vivo remyelinating potential although they are attractive candidates with demonstrated neurogenic potential (115). In females that received bone marrow transplants from male donors, male cells of neuronal phenotype could be found in the brain at autopsy (116). There are reports that the myelin synthesized by transplanted neural stem cells or bone marrow stromal cells is thicker than normal and results in a myelinating cell to axon ratio similar to peripheral myelin (117). This implies that remyelinated lesions will not be as compact as normal white matter, the functional implications of which are unknown. It is critical that a well defined population of human cells with the capacity to differentiate into oligodendrocytes be identified, characterized, and tested in multiple models for efficacy of remyelination.

PATHOGENIC ANTIBODIES DIRECTED AGAINST CNS ANTIGENS

Pathogenic CNS reactive antibodies likely contribute to both tissue damage in the MS lesion and to an environment that does not support tissue repair. The existence of pathogenic autoantibodies is well established in several peripheral neurologic syndromes including myasthenia gravis, Lambert Eaton syndrome, Guillain-Barre syndrome, and acquired neuromyotonia (118). The involvement of pathogenic autoantibodies in a particular disease is defined by several lines of evidence. Antibodies to a defined target should be present in the majority of patients with the disease. Induction of disease in animal models should be possible by immunizing animals with the target antigen, passive transfer of antibodies to the defined antigen, or transfer of antibodies from patients with disease. In diseases mediated by pathogenic antibodies, reducing serum antibody levels by plasma exchange or immunosuppression should lead to clinical improvement. Resynthesis of pathogenic autoantibodies should lead to a return of clinical symptoms.

The presence of antibodies to myelin oligodendrocyte glycoprotein (MOG) correlate with myelin breakdown in human MS and in primate EAE (119). Antibodies to MOG administered to animals with established EAE increases disease severity and shifts this predominately inflammatory model to a demyelinating disease (120). About 30% to 50% of active MS plaques contain a deposition of immunoglobulin and complement (121), suggesting a direct role of antibodies in disease progression. Plasma exchange, which reduces serum antibodies and complement, is effective in reducing clinical severity of fulminant MS exacerbations in approximately 40% of treated individuals (31).

REPARATIVE ANTIBODIES DIRECTED AGAINST CNS ANTIGENS

It may at first seem counterintuitive that autoantibodies can also promote tissue repair. The initial observation that autoreactive antibodies can enhance endogenous
remyelination was shown using the TMEV induced model of demyelination (21). Persistent TMEV infection leads to chronic immune mediated demyelination and progressive loss of motor function very similar to that observed in chronic progressive MS. Spontaneous remyelination of demyelinated spinal cord lesions, common in many other mouse strains, is limited in the SJL mouse strain. In general, less than 10% of the total demyelinated lesion area is remyelinated. The low background level of spontaneous repair makes this an excellent model for the study of strategies to promote endogenous remyelination.

Chronically infected SJL mice were immunized with spinal cord homogenate (SCH) in incomplete Freund’s adjuvant in an attempt to exacerbate demyelinating disease. SCH is a mixture of myelin and neuronal protein and lipid antigens. Surprisingly, rather than worsening the course of disease in virus infected mice, as would be conventionally predicted by any treatment that increased anti-CNS antibodies, especially antimyelin antibodies, the spinal cords of SCH immunized mice contained four to five times more remyelination than nonimmunized mice. Remyelination could also be enhanced to an equal degree by the passive transfer of antiserum (36) or purified immunoglobulin (122) from uninfected animals immunized with SCH. This demonstrated directly for the first time a beneficial role of the humoral immune response in promoting myelin repair.

Immunization with SCH induces a polyclonal antibody response directed against multiple CNS antigens. Further studies demonstrated that the reparative effect of polyclonal antisera can be replicated by the administration of monoclonal antibodies (mAbs). Hybridomas generated from the B-cells isolated from SJL mice immunized with SCH were screened in an antigen independent manner for the ability to promote remyelination in chronically demyelinated mice. Two mouse mAbs that enhanced remyelination were identified (123). Both mouse mAbs were IgMs that bound to oligodendrocytes when used for immunocytochemistry of cells in culture. Using oligodendrocyte binding as the initial selection criteria an additional four mouse IgMs and two human IgMs were identified that promoted CNS remyelination in vivo (40,124). The fact that all mAbs that promoted remyelination bound to the surface of oligodendrocytes suggested that the activity of these mAbs involved direct stimulation of the myelin producing cells (125).

The human remyelination promoting mAbs were identified from the Mayo Clinic sera bank, a unique collection of over 125,000 samples collected over 40 years. Serum derived human monoclonal IgMs (sHIgM) and serum derived human monoclonal IgGs (sHIgG) isolated from patients with monoclonal gammopathy, a relatively common condition characterized by high concentrations of monoclonal serum antibody, were screened for binding to the surface of rat oligodendrocytes. Six of 52 tested sHIgMs bound to oligodendrocytes, whereas none of 50 tested sHIgGs bound. Two of the human IgMs promoted remyelination in the TMEV model of chronic demyelination equal to that induced by human polyclonal immunoglobulin (40), an established therapy for many immune mediated disorders.

The remyelination promoting human mAbs are not pathogenic for the patients that synthesize the molecules. Neither patient presents with neurologic dysfunction despite having carried high levels of these mAbs for many years. Recombinant forms of the two human IgMs, designated rHIgM22 and rHIgM46, have been successfully synthesized (126). These mAbs can be made in large quantities sufficient for a clinical trial. Both recombinant human mAbs bind to oligodendrocytes and myelin (Fig. 1)
Figure 1  Antibody-mediated promotion of remyelination in the Theiler’s murine encephalomyelitis virus–induced model of demyelination. Mice of the SJL strain with chronic virus infection, demyelination, and clear neurologic deficits were treated with saline or a single 100 μg injection of a recombinant human monoclonal antibody, rHIgM22. Spinal cords were analyzed histologically five weeks later. Spinal cord cross sections were stained for the presence of myelin using p-paraprenylenediamine. Remyelinated axons are thinner than normal and therefore stain lighter. (A) An example of a demyelinated lesion from the spinal cord of an animal treated with saline. (B) An example of remyelination within a demyelinated lesion from the spinal cord of an animal treated with rHIgM22. rHIgM22 binds specifically to myelin and the surface of oligodendrocytes in unfixed tissue or cells in culture. (C) Phase contrast image of an unfixed slice of mouse cerebellum showing detail of one of the outer folia. (D) Corresponding immunofluorescence image to (C). rHIgM22, when used for immunocytochemistry specifically binds to myelinated tracts in the central white matter and granule cell layer. (E) Phase contrast image of human temporal lobe glia in cell culture. (F) Corresponding immunofluorescence image to (E). rHIgM22, when used for immunocytochemistry binds to the surface of oligodendrocytes. Abbreviation: rHIgM22, recombinant human monoclonal IgM.
from mice, rats, and humans and also elicit a substantial enhancement of remyelination in chronically demyelinated mice. rHIgM22 effectively crosses the blood–brain barrier (127) and is accumulated in CNS lesions in mice with chronic demyelination and is a very specific marker for white matter when used for immunocytochemistry on unfixed CNS tissue (128). Far more specific than the complete sera isolated IgM. rHIgm22 is effective at promoting remyelination in vivo at very low doses. A single 0.5 μg bolus administered intraperitoneally to a 20 g mouse effectively promotes remyelination. Anti-CNS mAbs may be a novel therapeutic treatment for human neurologic injury and disease.

Both remyelination promoting human mAbs appear to be naturally occurring polyreactive IgM autoantibodies. Human IgMs obtained from macroglobulinemia patients bound with high frequency to myelin antigens, suggesting that anti-CNS antibodies are common in the serum of individuals with no history of neurologic damage. Antibodies of this type are present in the serum of normal individuals and often bind to a variety of structurally unrelated, self and nonself antigens (129). These antibodies may represent a primordial aspect of the immune system that performs largely physiologic functions (130,131). Germline IgM antibodies may have developed as a mechanism of cell to cell communication in early multicellular organisms. One function may be to promote tissue repair. This IgM based system may have been later co-opted for immune surveillance during the evolution of an adaptive immune system. Immunization with SCH may mimic exposure of the immune system to CNS antigens that occurs following CNS injury.

CNS reactive antibodies may enhance not only myelin repair following demyelinating disease but also axon outgrowth following CNS trauma. Rodents immunized with SCH prior to spinal cord hemisection or optic nerve crush demonstrated enhanced axonal regrowth in both lesion models (38,132). Functional improvement was reported in the mice with spinal cord injury that received SCH immunization. The SCH immunization strategy in the axon injury studies were identical to that used in the remyelination studies and resulted in increased sera titers of myelin reactive antibodies. The sera from animals demonstrating the best axon regrowth correlated with the highest titers of myelin-reactive serum antibodies, which when assayed in vitro allowed axon outgrowth on immobilized CNS myelin, a substrate normally inhibitory to neurite extension.

It has been hypothesized that anti-SCH antibodies enhanced axon regeneration in vivo by blocking myelin associated inhibitors of axon outgrowth. However, the SCH antisera were reported to not contain elevated titers of antibodies to the known myelin inhibitors Nogo, myelin associated glycoprotein, and chondroitin sulfate proteoglycan. Unfortunately, the CNS reactive antibodies identified in animals with enhanced axon regeneration were not directly tested in vivo using a passive transfer protocol to prove with certainty that antibodies alone mediated the reparative response.

In vivo studies using the mouse mAb, IN-1, as a therapy for CNS trauma also support the premise that antibodies directed against CNS antigens can be reparative (133–135). In a number of model systems, IN-1 promotes axon regrowth and functional recovery following CNS injury (136). IN-1 is also an IgM that binds to oligodendrocytes and myelin (137) and is proposed to bind to and block the action of myelin antigens inhibitory to axon outgrowth exposed following tissue disruption (107,138). Despite the similarities of IN-1 to the human antibodies that promote remyelination the ability of IN-1 to enhance repair in models of chronic demyelination are untested.
GLATIRAMER ACETATE, AN ESTABLISHED TREATMENT FOR MS, MAY ACT VIA A HUMORAL IMMUNE RESPONSE

One of the established treatments for MS may act in part through a largely unrecognized antibody mediated repair mechanism. Glatiramer acetate (GA), also known as Copolymer-1 or Copaxone, is an immunogenic mixture of synthetic peptides that has been shown to be effective in reducing MS exacerbations, the appearance of new lesions by MRI, and the progression of disability (139,140). Despite experimental evidence that treatment with GA downregulates certain immune functions, the clinical use of GA indicates that other immune functions are stimulated by GA treatment, including the induction of T-cell activation and anti-GA antibody synthesis. All MS patients treated with GA develop antibodies to GA, but the characteristics of these antibodies remain largely unexplored. There is a correlation between the presence of antibodies against GA and the therapeutic efficacy of GA in an individual. Patients who remain relapse-free after two years of GA treatment have statistically higher titers of anti-GA antibodies than those who develop relapses (141). An additional indication that GA stimulates the immune system is the localized swelling and occasional hypersensitivity reactions in response to GA. Since most therapy for MS is designed to reduce immune activity, it is unexpected that a compound which elicits a strong immune response would be therapeutic.

A study of the effect of passively transferred GA reactive T-cells or anti-GA antibodies on disease in chronically demyelinated mice raises the intriguing possibility that the antibody response in GA treated patients is beneficial by facilitating the repair of demyelinated lesions (142). Immunization with GA alone or with adjuvant, or the transfer of GA reactive lymphocytes did not alter the extent of spinal cord demyelination or remyelination. In contrast, spinal cord remyelination was increased by more than twofold following the passive transfer of affinity purified polyclonal antibodies to GA over a five week period. Anti-GA antibodies were isolated from the sera of uninfected SJL mice immunized with GA and adjuvant. Anti-GA antibodies share a few characteristics with other remyelination promoting antibodies. In sections of spinal cord anti-GA antisera bound to oligodendrocytes, perivascular infiltrating cells, astrocytes, and neurons, while in glial cultures anti-GA antisera bound to early stages of the oligodendrocyte lineage and microglia. mAbs to GA generated in rodents cross-react with the myelin antigen, MBP (143,144) and antisera to MBP promotes remyelination (145). GA or MBP reactive lymphocytes reduce secondary neuronal degeneration following experimental optic nerve damage (146) also linking the property of antimyelin reactivity to the protection of axons.

An apparent paradox in this study is that adoptive transfer of antibodies to GA promoted remyelination, yet active immunization with GA did not. In fact, immunization of mice with high-dose GA increased lesion load, suggesting that GA has multiple effects in vivo and that the positive influence of the antibodies to GA was overridden by other effects of GA immunization. GA suppresses T-lymphocyte activity in a relatively nonspecific manner (147–150). Since T-lymphocytes are essential for controlling TMEV even during late disease (42), antiviral immunity may have been depressed by immunization with GA, resulting in increased viral pathogenesis and lesion exacerbation. Increased virus antigen expression and decreased antiviral antibody titers were reported in the GA treated mice.
MECHANISM OF ANTIBODY-MEDIATED CNS REPAIR

Since all of the remyelination promoting mAbs bind to oligodendrocytes or myelin, it seems reasonable to suggest a direct effect on the recognized cells. As a group, remyelination promoting mAbs bind to a limited number of antigens on the surface of live oligodendrocytes. The surface antigens bound by several of these mAbs have been characterized and are generally lipid or carbohydrate in nature (125,151). mAbs that bind to the oligodendrocyte specific antigens galactocerebroside, sulfatide, and myelin/oligodendrocyte specific protein can elicit biochemical and morphological changes in glial cells (152), which are preceded by an mAb induced calcium influx (153). Similar transient calcium fluxes were observed in a subpopulation of astrocytes and oligodendrocytes following the addition of remyelination promoting mAbs to the culture media (154). There is a high degree of correlation between the ability of a mAb to promote remyelination and its ability to stimulate calcium influx suggesting a potential connection between these two phenomena. Remyelination promoting mAbs may act directly by binding to and inducing a signal in oligodendrocyte progenitors (154) or protecting oligodendrocytes from stressor molecules (155) or indirectly by binding to astrocytes and inducing the release of soluble factors. Myelin binding mAbs may also enhance myelin repair through other indirect mechanisms. mAb binding to injured oligodendrocytes and their progenitors that are incapable of myelination may enhance the opsonization and clearance of these cells and myelin debris by macrophages (156). Large numbers of macrophages are often observed within demyelinated lesions and phagocytosis of myelin debris may be an important prerequisite to efficient remyelination. It remains to be determined whether remyelination promoting mAbs utilize one or several of these mechanisms (Fig. 2).

The promotion of remyelination by mAbs has been demonstrated in both immune and nonimmune mediated experimental models of demyelination (33,157). The therapeutic effectiveness of mAbs in multiple experimental models indicates that the underlying mechanism is not a modulation of model specific pathogenesis, but is likely a fundamental physiologic stimulation of a reparative mechanism. The human recombinant mAb, rHIgM22, does not appear to act through an immunomodulatory mechanism (158). Treatments that induce immune suppression in chronically TMEV infected mice normally leads to reactivation of virus as manifested by increased viral titers in the CNS. Administering rHIgM22 does not alter the immune response in mice with acute virus infection or alter the level of virus specific RNA or the number of virus antigen positive cells in the spinal cord of chronically TMEV infected mice. The disease course of established EAE is unaltered by treatment with rHIgM22. Together, these studies establish that virus clearance is not a prerequisite for mAb enhanced remyelination to proceed. Given the hypothesis that MS involves chronic immune stimulation possibly as a result of an infectious agent, these observations suggest that mAb mediated remyelination strategies may be effective in MS.

Antibodies that bind to the neuronal membrane have also been shown to directly induce signals in neurons and alter their morphology. mAbs that bind to the ganglioside GMI suppressed neurite outgrowth in vitro and in vivo (159,160), whereas anti-idiotypic antibodies to GMI induced neurite extension in hippocampal and dorsal root ganglion neurons (161). The binding of mAbs to the ganglioside GD3 (R24) or to a cerebellar granule cell surface protein (TAG-1) induced activation of the Src family kinase Lyn and resulted in similar changes in cell protein tyrosine phosphorylation. Reducing the concentration of membrane
GD3 by removing surface carbohydrates from ceramides eliminated mAb mediated signaling through both GD3 and TAG-1 (162,163) suggesting that membrane glycosphingolipids may be required for GPI-linked protein mediated signaling.

Critical differences in the efficacy of remyelination promoting IgMs and their monomeric and smaller fragments have been described (164). Studies utilizing neuronal ganglioside binding mAbs also support the importance of the IgM isotype in eliciting a biologic response (165). High affinity anti-GT1b and anti-GDI IgGs were only successfully isolated from mice that lacked endogenous complex gangliosides. The antiganglioside IgGs attenuated CNS myelin inhibition of neurite extension presumably by interfering with access to myelin antigens, but did not inhibit neurite extension on their own. Only after complexing the IgGs into multivalent molecules did the mAbs block neurite extension directly, similar to the effect of an anti-GT1b IgM. Antiganglioside antibodies are associated with a number of human neuropathies. The key characteristic of an mAb that determines whether it can elicit a signal appears to be whether the mAb can cluster a sufficient number of molecules within the cell membrane. A multivalent molecule such as the large pentameric IgM

**Figure 2** Potential mechanisms of action for remyelination-promoting antibodies. Reparative antibodies have been demonstrated to bind to myelin and oligodendrocytes in culture and target to and accumulate at sites of demyelination in vivo. Within the demyelinated lesion remyelination-promoting antibodies may directly bind to surface receptors on oligodendrocytes, enhancing their proliferation, differentiation, or survival. Remyelination-promoting antibodies may act indirectly by binding to and aiding in the clearance of myelin debris and injured or dying oligodendrocytes incapable of myelination. mAbs may bind to other cell types within the demyelinated lesion, such as astrocytes or immune cells, inducing the synthesis of soluble factors that drive remyelination. mAbs may alter a lesion environment that is unsupportive of remyelination by potentiating oligodendrocyte recognition of and binding to demyelinated axons.
may act by bringing together disparate signaling molecules or increase the effective avidity by clustering low affinity receptors and ligands to a critical level. This may be why most mAbs that elicit a biologic response are IgMs.

REMYELINATION PROMOTING mAbs TARGET THE DAMAGED CNS

There is ample evidence in human MS that the blood–brain barrier is open during acute exacerbations, but the blood–brain barrier may also be open in lesions that remain clinically silent. A remyelination promoting mouse IgM does cross the blood–brain barrier in animals with CNS demyelination. A detailed pharmacokinetic analysis examined the distribution of radiolabeled mouse IgMs in TMEV infected and uninfected mice following intravenous injection (166). IgMs did not enter the CNS of uninfected mice, but both a remyelination promoting IgM and a nonremyelination promoting control IgM readily entered the brain and spinal cord of infected mice. Of particular importance the control IgM was cleared from the CNS by 24 hours following injection, whereas the remyelination promoting IgM was detectable for as long seven days. The remyelination promoting IgM specifically bound to oligodendrocytes and myelin debris within the demyelinated lesions and was not concentrated in areas of morphologically normal CNS. It appears that remyelination promoting mAbs can directly target in vivo oligodendrocyte antigens in damaged tissue. In contrast, control IgMs never found a target and were promptly lost from the CNS.

The recombinant human remyelination promoting mAb, rHIgM22, also entered the CNS and was concentrated in demyelinated lesions in TMEV infected mice (127). Sensitive MR imaging was used to track the movement of rHIgM22 to the CNS following peripheral injection. Four weeks following TMEV infection SJL mice were given an intravenous injection of biotinylated rHIgM22. Four hours later strepavidin complexed to a particulate MRI contrast material, ultra small superparamagnetic iron oxide (USPIO) was administered intravenously. Localized concentrations of strepavidin-USPIO caused by clustering around the pentameric IgM appear as hyperintense areas on T1-weighted images. High T1 signal areas were observed in the brain stem of rHIgM22 treated virus infected mice, but not observed in the corresponding images of the similarly demyelinated animals without the addition of rHIgM22. Postcontrast T1 signals were not recorded in uninfected control mice or infected mice injected with a biotinylated control IgM that does not bind to the CNS or promote remyelination. It appears that rHIgM22 enters and accumulates within the demyelinated CNS, but does not enter the normal CNS.

rHIgM22 mediated lesion repair has also been followed in the TMEV mediated model of MS by a quantitative MRI analyses of lesion volume (127). Individual chronically demyelinated mice were imaged by MRI before receiving a single treatment of either rHIgM22 or saline and five weeks later. The mean demyelinated lesion load between the two groups was not significantly different before mAb treatment. However, five weeks later the mean lesion load of the rHIgM22 treated mice was significantly smaller. Mean lesion load decreased by 40.6% in the rHIgM22 treated group, whereas lesion load increased by 13.6% in saline treated animals. Lesion volume decreased in every one of 13 mice treated with rHIgM22, whereas lesion volume increased in seven of eight mice treated with saline. Following the second imaging session, animals were examined histologically. Areas of demyelination were smaller and less pronounced and remyelination corresponded with a localization of MR signal reduction.
THE CHALLENGE OF BALANCING INFLAMMATION FOR REGENERATION

A long held dogma in the MS field is that immune activation, both cellular and humoral, exerts an overwhelmingly deleterious role and must be suppressed for effective therapy. However, it is becoming increasingly clear that the immune system can also be protective to the injured CNS. As the understanding of MS and the basis of the observed limited repair increases, the arsenal of potential regeneration therapies will continue to expand. To correctly assess these emerging therapies clinical trials must be designed to measure the extent of tissue repair and axonal preservation (167), not merely changes in inflammation. Therefore, technologies must evolve in tandem to noninvasively characterize MS lesions prior to treatment and to directly measure the degree of lesion repair or a surrogate marker of repair.

The immune system is intimately involved with the progression of MS and potentially its reversal. The overall immune balance within a lesion determines whether a path of repair or disease will evolve. How existing treatments for MS shift this inflammatory balance need to be carefully studied. Nonspecific anti-inflammatory therapies may need to be abandoned. Combined therapies designed to control specific aspects of inflammation and encourage regenerative endogenous repair are likely the future of treatment. A major therapeutic goal should be to protect axons long enough for remyelination to make that protection permanent.

Of the present therapeutic choices, remyelination promoting mAbs may be the best single treatment approach. mAb therapy may be combined with glial cell transplantation in patients lacking a sufficient number of myelinating cells. Reparative CNS binding mAbs represent a new class of therapeutics for diseases such as MS, spinal cord injury, neurodegeneration, and stroke. mAb-based therapeutics offer a specificity of binding and potential of action not possible with other reagents. Human mAbs are likely to be minimally antigenic when administered systemically, for Abs are normally present in the circulation. Human mAbs have a number of advantages as therapeutics in contrast to administering an antigen to induce an individual to synthesize their own CNS binding antibodies, which may produce unpredictable immune reactions across the population.

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Axonal Injury in Multiple Sclerosis

Gerson A. Criste and Bruce D. Trapp
Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio, U.S.A.

INTRODUCTION

The pivotal role of axonal injury in the pathogenesis of multiple sclerosis (MS) has become a major focus of MS research in recent years. Axonal injury, considered to be a late phenomenon at one time, is now recognized as an early event in the progression of MS pathology. There is a body of evidence from histopathologic, as well as contemporary neuroimaging modalities like magnetic resonance imaging (MRI) and spectroscopy, that axons play a crucial and dynamic role during the evolution of MS pathology and the development of clinical disability. The mechanism of axonal injury in MS, however, remains diverse and speculative. Although generally considered to be sequelae of inflammatory demyelination, the limited success of immunotherapy to provide a halt to progressive disability has diverted our attention to other possible mechanisms. The possibility that axonal injury can be partly reversible, at least in the acute phase, has provided an impetus to institute early therapy. The finding that diffused, irreversible axonal transection occurs early in the course of this complex disease has underscored now, more than ever before, the need for axonal neuroprotection.

While these new concepts make MS even more complex, it provides a new challenge and opportunity for those working on MS, which will later translate to novel therapeutic possibilities for MS patients. This chapter reviews current data on axonal pathology in MS.

AXONAL PATHOLOGY IN MS LESIONS

Recent studies using contemporary technology, such as MRI and confocal microscopy, demonstrated that axonal transection begins at disease onset and cumulative axonal loss provides the pathologic substrate for the progressive disability, which most long-term MS patients experience. Moreover, postmortem studies have shown that several histopathologic abnormalities including axonal loss can be detected in the normal appearing white matter (NAWM) (1) and cortical gray matter (2) of patients with MS, suggesting a more diffuse pathology than previously thought.
Early Reports

Although a somewhat controversial subject, axonal pathology was mentioned in the early literature on MS. These reports include descriptions of axonal swellings, axonal transection, Wallerian degeneration, as well as discussions regarding the functional consequences of such pathology (3). In their classical works, both Charcot and Marburg (4,5) described MS pathology in terms of demyelination and reactive gliosis. However, they also emphasized the relative sparing of axons in the lesions. In 1936, Putnam (6) reported a 50% loss of axons in MS lesions from 11 patients. In contrast, Greenfield and King (7) reported normal axon densities in more than 90% of MS lesions from 13 patients in the same year. The differences between these works were suggested to result from more sensitive axon staining in the latter. Subsequently, the axonal component of MS pathogenesis received less attention, and the question regarding axonal damage in MS remained unclear for a long time.

Current Evidence

Axonal Transection Occurs During Early Stage of MS

Amyloid precursor protein (APP), which is present in axons at levels not normally detected by immunohistochemistry, is transported by fast axonal transport (8). Immunohistochemical detection of axonal APP indicates functional impairment of the labeled axons. Ferguson et al. (9), described APP accumulation in axons located in active MS lesions and at the border of chronic active MS lesions. Some APP immunoreactive structures exhibited the morphology of terminal axonal swellings, suggesting axonal transection. The number of APP labeled axonal swellings correlated with the degree of inflammation in the lesions (9). Using confocal microscopy and computer-based three-dimensional reconstruction, extensive axonal transection was demonstrated in cerebral white matter MS lesions from 11 patients with disease duration ranging from 2 weeks to 27 years (1). Axonal ovoids were identified as terminal ends of transected axons in the confocal microscope (Fig. 1), and the degree of inflammation in the lesions was characterized by the presence of activated macrophages and microglia. Active lesions contained over 11,000 terminal ends per mm³, the edge of chronic active lesions contained over 3000 terminal ends per mm³, and the core of chronic active lesions contained an average of 875 terminal ends per mm³. In contrast, less than one transected axon was found per mm³ in control white matter. Together, these data demonstrate a positive correlation between axonal transection and degree of inflammation in cerebral white matter MS lesions undergoing demyelination. The presence of axonal ovoids in patients with short disease duration demonstrated that axonal transection begins at an early stage of MS (1).

Axonal Loss Is Seen in NAWM

It is well established that axons once severed will undergo relatively rapid Wallerian degeneration distal to the site of transection. Unlike axons, central nervous system (CNS) myelin can persist for a long time after proximal fiber transection. Histologically, such remaining myelin sheaths may appear as empty tubes or as degenerating ovoids. Despite this microscopic pathology, however, the white matter may appear normally grossly and with conventional neuroimaging studies.

Immunohistochemical evidence suggestive of Wallerian degeneration, such as discontinuous staining of axonal neurofilaments and presence of terminal axonal
ovoids, has been demonstrated in NAWM from MS brains (1). The extent of axonal loss in this region has been addressed quantitatively. Ganter et al. (10) working in areas without plaque reported reductions in axonal density by 19% to 42% at the lateral corticospinal tract of MS patients with lower limb weakness. Lovas et al. (11) compared axonal density in lesions and NAWM from the cervical spinal cords of secondary progressive MS (SPMS) patients. The average reduction in axonal density in lesions from lateral and posterior columns was 61%. In NAWM, however, the average decrease in axonal density was as much as 57%. They also noted that axons with diameters smaller than approximately 3 μm were more affected than larger axons. In a study that accounted for both decreased axonal density and changes in tissue volume, total axonal loss in the corpus callosum of MS patients with disease durations between 5 and 34 years and various degree of functional impairment averaged 53% (10). Note, however, that in the same material, the reduction in axonal density was only 34%, emphasizing the need to consider both tissue volume and axonal density to properly assess the degree of total axonal loss. These studies suggest that white matter may appear normal upon immunohistochemistry for myelin, or on MRI scans, but may still exhibit a considerable axonal dropout, especially in chronic patients with long disease duration.

**Figure 1** Axons end in large terminal ovoids (arrows) indicating axonal transection during demyelination. *Source: From Ref. 1.*
Wallerian degeneration in NAWM has been observed by immunohistochemistry in an MS patient with short disease duration (12). The patient succumbed to a fatal brain stem lesion just after a nine-month-history of relapsing–remitting MS (RRMS) with few permanent neurologic signs. Demyelinated lesions were not found in the spinal cord postmortem. However, the ventral column of the spinal cord, containing tracts projecting from the brainstem lesion, exhibited a 20% axonal loss. Microscopy revealed myelin ovoids and signs of myelin degradation by activated microglia, characteristic of Wallerian degeneration (Fig. 2). Since much of the myelin remains, these can be “invisible lesions” as far as MRI and immunostaining for myelin are concerned.

Neuronal Pathology Is Seen in MS Cortex

In addition to the more commonly described white matter locations, MS lesions can also involve gray matter (13,14). However, the histopathological features as well as the clinical significance of such lesions are not completely understood. MS lesions in the cerebral cortex are less obvious than white matter lesions on conventional T2-weighted images (15). Gray matter lesions are also difficult to detect macroscopically and histologically. Histologically, the frequency of cortical lesions has often been underestimated. Recently, Kidd et al. (15) demonstrated that the use of gadolinium enhancement resulted in an increased detection of cortical lesions on MRI scans by 140%. Twenty-six percent of these enhancing lesions arose within or adjacent to the cerebral cortex. This study also suggested that conventional MRI under-reports the presence of cortical lesions, when compared with neuropathological analysis.

In a recent postmortem study on MS brains using immunohistochemistry and confocal microscopy, the characteristics of gray matter lesions were described (2). Significant neurite transection and apoptotic loss of neurons were seen. Interestingly, compared to its white matter counterpart, inflammation is reduced in these lesions. Gray matter lesions contained fewer inflammatory cells, no perivascular cuffs, and consisted mainly of reactive microglia. Of interest is the distribution of T-lymphocytes in these lesions since T-cells have been proposed to take a central role in the pathogenesis of MS. Bo et al. (16) studied the density of lymphocytes among MS lesions

Figure 2 Wallerian degeneration in normal-appearing white matter from a patient diagnosed with relapsing–remitting multiple sclerosis for nine months. In both cross section (A) and longitudinal section (B), myelin ovoids lacking axons (arrows) were detected. In longitudinal section (B), these myelin ovoids often lay in rows. Source: From Ref. 12.
and found that the highest density of lymphocytes was found in MS white matter lesions. Fewer T-cells were detected in cortical lesions that extended through both white and gray matter. The lowest number of T-cells was detected in intracortical demyelinated lesions which was equal to the lymphocyte density in nondemyelinated cerebral cortex within the same tissue block.

It has been hypothesized that injury to neurons in cortical and subcortical MS lesion is responsible for the cognitive dysfunction many MS patients experience (15,17). In fact, executive and cognitive functional deficits arise in 40% to 70% of these patients (18–21). Increased knowledge, regarding mechanisms of neuronal damage, in cortical MS lesions will contribute to the understanding of the functional significance of such lesions.

**MECHANISM OF AXONAL INJURY IN MS**

The pathophysiology of axonal injury in MS is poorly understood. It is possible that several different mechanisms of axonal degeneration occur at different stages of the disease. Elucidating the cellular and molecular mechanisms of axonal loss in MS will influence the development of future neuroprotective therapies.

The correlation between inflammatory activity and number of transected axons in cerebral MS lesions support the hypothesis that inflammatory demyelinating environments injure axons (1,9). At later stages of MS, extensive axonal loss and progression of disability occur in the absence of overt inflammatory activity. This suggests that mechanisms other than inflammatory demyelination contribute to axonal degeneration. Recently, it was proposed that abnormal expression of sodium channel subtypes in response to demyelination may render axons vulnerable to degeneration, raising the possibility that MS may involve an acquired channelopathy (22). More importantly, a number of genes coding for myelin related proteins such as myelin-associated glycoprotein (MAG), proteolipid protein (PLP), and 2, 3-cyclic nucleotide 3-phosphodiesterase (CNP) are being studied in relation to axonal pathology. It is postulated that the lack of trophic support from myelin or myelin forming cells may cause degeneration of chronically demyelinated axons (23,24).

**Genetics and Susceptibility to Axonal Injury**

The disease course of MS is highly variable between patients. Both environmental factors and genetic predisposition contribute to susceptibility and clinical heterogeneity of the disease (25,26). Current evidence indicates that interactions between multiple genes influence the outcome of MS in individual patients (27). For example, genetically determined response of various tissue components to inflammation could influence the development of tissue damage in MS. Data suggesting a genetic component in the axonal response to inflammatory demyelination is provided from Theiler’s murine encephalomyelitis virus (TMEV) disease, a virus induced model of inflammatory CNS demyelination. Infected animals with susceptible genetic background develop neurological impairment and pathological changes comparable to those in MS (28,29). Infected SJL/J mice develop chronic demyelination, neurological deficits, and extensive loss of axons in the spinal cord (30). Interestingly, the mice lacking the major histocompatibility complex (MHC) class I in a strain, usually resistant to TMEV induced disease (C57BL/6 × 129 mice), develop a similar distribution and extent of demyelinated lesions as SJL/J mice after infection but no functional disability was observed. It was proposed that absence of overt neurologic
dysfunction despite demyelination results from increased sodium channel densities and the relative preservation of axons. In contrast, C57BL/6 × 129 mice, lacking MHC class II, developed various neurological signs such as stiffness and paralysis, and exhibited axonal pathology and axonal degeneration in spinal cord white matter four months after infection (31). The neurologic symptoms in these class II-deficient mice were suggested to result from axonal injury. These results indicate that MHC class I is involved in the process leading to axonal damage and highlights the possible role of genetic influence on the development of axonal degeneration and neurological symptoms during inflammatory demyelination.

In light of the ongoing studies about mechanisms leading to axonal injury in MS, genes encoding for trophic factors that are involved in neuroprotection and repair are just as important as immune-related genes that are thought to be responsible for the pathology. In this context, ciliary neurotrophic factor (CNTF) is an interesting candidate to possibly abate immune-mediated axonal injury in MS. One study found a correlation between the presence of CNTF null mutation and earlier onset of MS symptomatology (32). This suggests that axonal loss, which is the basis of disability, may be accelerated in these individuals by lack of CNTF’s trophic support of neurons and oligodendrocytes following an inflammatory attack, which may be crucial for survival and recovery. Although another study did not find a correlation of CNTF genotype and onset, course, and severity of disease (33), the results of Geiss et al. (32) are in accordance with the observations made in experimental allergic encephalomyelitis (EAE) in CNTF knock-out mice. After induction of myelin oligodendrocyte glycoprotein, CNTF−/− mice with experimental autoimmune encephalomyelitis showed a significantly earlier disease onset and a delayed recovery from relapses (34).

Considering the genetic component in MS, the variation in individual susceptibility, and the differences in clinical course between patients (25–27), it is possible that genes involved in axonal responses to demyelination influence the outcome of MS in susceptible individuals. Knowledge of the genetic events leading to axonal injury and eventual disability in MS will create new opportunities to prevent, treat, and cure this terrible disease.

**Axonal Injury and Inflammation**

Current knowledge suggests that MS is a primary inflammatory demyelinating disease of the CNS. Moreover, several lines of evidence indicate that disease activity reflects CNS inflammation, even when the disease is subclinical (35). For example, most RRMS patients exhibit progressive brain atrophy and persistent inflammation, as identified by gadolinium-enhanced lesions on MRI scans, regardless of the presence of clinical symptoms, and will also exhibit progressive disease on subsequent MRI examinations (36–38). Since axon pathology and frequency of transected axons in MS lesions correlate with the degree of inflammation (1,9), early axonal transection might occur due to vulnerability of demyelinated axons to inflammation. Indeed, the inflammatory microenvironment contains a variety of substances that could potentially injure axons, such as proteolytic enzymes, cytokines, oxidative products, and free radicals produced by activated immune and glial cells (39). Recently, data indicating that cytotoxic CD8+ T-cells can mediate axonal transection in active MS lesions were provided in MS tissue (40), EAE mice (41), and in vitro (42). Another observation is that treatment with the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid/kainate glutamate receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzoquin resulted in increased oligodendrocyte survival and
reduced axonal damage in EAE. This suggests that excitotoxicity mediated by glutamate is involved in tissue damage in acute lesions (43). In addition, inflammation may affect energy metabolism of axons directly or indirectly (44). Inflammatory intermediates may act directly on the mitochondria, and local inflammatory edema may interfere with blood supply and supposedly induced an ischemic mechanism of axonal degeneration. This mechanism is further discussed in later part of this chapter.

Inflammation causing irreversible tissue damage is a major factor behind accumulating axonal pathology at early stages of MS. Therefore, aggressive anti-inflammatory treatment during RRMS may also have, in addition to effects on the inflammation, indirect effects in preventing axonal injury.

Myelin-Related Axonal Loss

The past decade has seen a deeper understanding of the intricate interdependence between the myelin forming cells and its associated neuron. For example, the neuron through axonal neuregulin has been found to control the proliferation and particularly the survival of oligodendrocytes and Schwann cells, ensuring a good match between the axon surface area requiring myelination and the number of surviving myelinating cells (45). Likewise, the myelin-forming cell also has a profound influence on axons morphology and physiology (46). Studies in Trembler and control mice demonstrated that myelinating Schwann cells affect axonal diameter, neurofilament phosphorylation, cytoskeletal organization, and axonal transport rates. Oligodendrocytes have a similar effect at the CNS (47). It follows, therefore, that diseases which affect myelin forming cells might influence the underlying axons as well.

Dysmyelinating diseases (in which myelin form abnormally) are easily associated with axonal changes. Charcot-Marie-Tooth neuropathy type 1 (CMT1) is a genetically heterogeneous group of chronic dysmyelinating peripheral neuropathies. Mutations affecting the myelin genes, peripheral myelin protein 22, protein zero, and connexin-32 account for most CMT1 cases (48). However, the dysmyelination does not fully account for the neurologic symptoms in CMT1. It turns out that the main contributor to clinical progression is the axonal loss, as determined by measurements of nerve conduction amplitudes and motor unit numbers (49). As in MS, this may be related to abnormal glial–axonal interactions (50). Thus, axonal degeneration may be a final outcome common to a wide variety of myelin diseases (51).

Myelin-forming cells also support axon in less obvious ways. There is compelling evidence that axonal pathology can result from mutations in myelin genes that cause little or no myelin abnormality. This corroborates the concept that myelin-forming cells support axons by way of trophic factors and molecules to maintain axonal homeostasis throughout a patient’s lifetime. In this section we focus on several molecules that may mediate such function.

MAG, a member of the immunoglobulin gene superfamily with receptor- or ligand-like properties (52–54), is enriched in the adaxonal membrane of myelin internodes (55–57). MAG inhibits neurite outgrowth (58,59) and causes growth cone collapse in vitro (60), suggesting that it can modulate the axonal cytoskeleton. In MAG-deficient mice, myelination progresses as in wild type animals with normal amounts of myelin. However, from the age of five weeks, progressive axonal atrophy including reduced axonal caliber, reduced neurofilament spacing, reduced neurofilament phosphorylation, and Wallerian degeneration was observed. The findings indicate that MAG has direct or indirect long-term modulating effects on the cytoskeleton via axonal kinases or phosphatases (55).
Recently, mice with a disrupted gene for a key enzyme in the biosynthesis of complex gangliosides, GM2/GD2 synthase, were generated. These animals develop decreased central myelination, axonal degeneration in both CNS and PNS, and demyelination in peripheral nerves (61). The neurodegenerative features were similar to those observed in sciatic nerves of MAG deficient mice as described above (55). Interestingly, the ganglioside-deficient animals also have reduced MAG expression in the CNS. These studies raise the possibility that complex gangliosides are endogenous binding partners for MAG, playing a role in maintenance of axons and myelin sheaths (61).

The X-linked PLP1/Plp gene encodes PLP1 and its minor isoform, DM20. PLP1 and DM20 are four-pass membrane proteins that together constitute over 50% of CNS myelin protein. PLP, a major structural protein of compact CNS myelin, has been proposed to stabilize the intraperiod line of central myelin sheaths (62). Mutations, deletions, or duplications involving the PLP gene cause Pelizaeus Merzbacher disease (PMD) and spastic paraplegia of varying severity in humans (63–65). In the jimpy mouse, PLP mutations result in premature oligodendrocyte death and dysmyelination (66). Many of these phenotypes, however, are considered “gain of function” effects due to toxicity of misfolded proteins encoded by the mutated genes. In contrast, the PLP null-mutant mice are still competent to myelinate CNS axons of all calibers and to assemble compacted myelin sheaths. Ultrastructurally however, the electron-dense “intraperiod” lines in myelin remain condensed, correlating with its reduced physical stability. From the age of six weeks, PLP-deficient mice exhibit focal axonal swellings with dense bodies and mitochondria in CNS regions containing mainly small diameter axons (67). The accumulation and distribution of organelles and neurofilaments in axonal swellings indicate impairment of retrograde axonal transport. Late onset axonal degeneration and progressive neurological disability is also seen in transgenic mice that moderately overexpress the PLP gene (68).

Although axonal degeneration in the Plp knockout mouse is late in onset, mice deficient in both PLP/DM20 and MAG develop a more severe CNS axonopathy, in which clinical signs begin by four weeks of age (69). It is not clear why the PLP/MAG double-knockout mouse is so severely affected, insofar as the absence of MAG alone has a relatively subtle phenotype (70).

The findings in the PLP knockout mice led to studies to evaluate axonal integrity in patients with mutations in PLP1. Garbern et al. (71) reported a length-dependent axonal degeneration in the absence of demyelination and inflammation in patients with null PLP1 mutations.

Recently, another myelin protein was implicated in axonal survival. CNP is expressed in oligodendrocytes and Schwann cells and is the earliest known myelin-specific protein to be synthesized. Unlike other oligodendrocyte protein, CNP is essential for axonal maintenance but less likely to contribute to myelin assembly (72). The CNP1 gene encodes two isoforms of 46 kDa and 48 kDa. CNP accounts for approximately 4% of all CNS myelin protein and is distributed throughout the cell soma (73) and in noncompacted regions of myelin: the inner mesaxon, paranodal loops, and Schmidt-Lantermann incisures (74,75). CNP has been shown to hydrolyze 2,3-cyclic nucleotides into their 2-derivatives, but, because 2,3-cyclic nucleotides have not been found in the brain, the function of CNP remains obscure (51).

Lappe-Siefke et al. (72) generated a mouse that lacks CNP1 expression. Surprisingly, myelin assembly was not visibly affected. Myelin was abundant and of regular thickness; normal periodicity was maintained and the structure of the
paranodes where CNP is normally localized is well preserved in many fibers. However, behavioral analysis showed that, at about four months of age, the mice developed motor deficits that progressed with age and subsequently died prematurely. This prompted further histological analysis that revealed late-onset axonal pathology characterized by abnormal axonal swellings and degeneration of many axons, clearly not related to dys- or demyelination.

The data from the myelin protein gene null mice show the dual roles of the oligodendrocyte: first, the formation of the myelin sheath and second, maintenance of the underlying axon through individual myelin molecules. The findings in the CNP deficient mouse indicate that these two functions can be uncoupled—that oligodendrocytes support axons independent of myelin function (72). This finding is relevant to MS where white matter lesions are associated with axonal injury and the causal relationship of inflammation, demyelination, and axonal damage are difficult to establish. In addition, since this model lacks inflammation, it clearly departs from the previously held notion that axonal injury is a bystander effect of inflammatory demyelination and suggests that functional oligodendrocyte pathology can contribute to axonal loss and progressive neurologic disability in MS.

Mitochondrial Component of Axonal Injury

Recent evidences suggest a hypoxia-like metabolic injury as a pathogenetic component of axonal injury in MS. Although this model was largely derived from studies of white matter injury in models of ischemia and neurotrauma, recent observations suggest that such mechanism operates in inflammatory brain lesions such as MS as well (76). In this model, ischemia leads to adenosine triphosphate (ATP) depletion. The resulting energy crisis impairs the function of ATP dependent ion channels (e.g., Na–K ATPase, Na–Ca ATPase) leading to an increase in intracellular Na concentration. Accumulation of axoplasmic Na through noninactivating Na channels, together with membrane depolarization, promotes reverse Na–Ca exchange and axonal Ca overload. Ultimately, the pathologic increase in intracellular Ca drives Ca-dependent enzymes to damage the axon.

It is not hard to see how this mechanism applies to MS especially if we look at this concept in the context of imbalance between the supply of cellular energy and demand. First, let us look at the supply side of the equation. Astrocytes (77), activated microglia, and macrophages (78, 79) in the CNS release substantial amount of nitric oxide (NO) in MS lesions. One mechanism of the toxic action of NO is the impairment of mitochondrial function leading to a state of energy failure. Indeed, exposing central white matter to NO causes ATP depletion and irreversible injury (80). Moreover, mitochondrial dysfunction has been implicated in a very recent study based on microarray analysis of postmortem MS motor cortex. This analysis found a decrease in nuclear encoded mitochondrial genes from four of the five complexes involved in the mitochondrial respiratory chain (81). This raises the possibility that there may exist inherent defects in these organelles in MS which may further compromise energy production capacity.

In addition to this metabolic disturbance, microvascular pathology also contributes a major role in the hypoxic MS pathology (44). Edema within inflammatory lesions leads to focal disturbance of microcirculation with subsequent ischemia. Such a mechanism may play a more important role in the pathogenesis of axonal damage in anatomical locations of the CNS where the room for tissue expansion is limited like the spinal cord (82) and the optic nerves. Moreover, inflammatory damage to
the vessel walls can lead to activation of the clotting cascade resulting in local microvascular thrombosis (83) and ischemic injury to the axons similar to a stroke.

These problems on the supply side of the energy equation is aggravated by the unfavorable energy demand in demyelinated axons. In the absence of myelin, the efficiency of salutatory conduction of nerve impulse is lost. To restore conduction, nerve fibers compensate by expressing Na channels along the length of the naked internodal axolemma. However, propagation of action potential under this circumstance exacts a high price in ion movements and increases demands on energy supplies as ion gradients are restored by ATP consuming pumps. Taken together, the unfavorable cellular energy supply and overwhelming demand for such energy in MS leads to the final catastrophic increase in intracellular Ca, which leads to axonal destruction.

Recent reports on the beneficial aspects of Na channel blockers in attenuating axonal pathology in animal models of MS probably reflect the relevance of the aforementioned hypothesis. Bechtold et al. (84) very recently described how flecainide, a Na channel-blocking agent, reduces axonal degeneration in an experimental model of MS, chronic relapsing experimental autoimmune encephalomyelitis (CR-EAE). Rats with CR-EAE were treated with flecainide or vehicle from either three days before or seven days after inoculation (dpi) until termination of the experiment at 28 to 30 dpi. Morphometric examination of neurofilament-labeled axons in the spinal cord of CR-EAE animals showed that both the flecainide treatment regimens resulted in significantly higher numbers of axons surviving the disease compared with controls. This corroborates earlier reports by Lo et al. (85), in the success of using another Na channel blocker, phenytoin, in the amelioration of spinal cord axonopathy and preservation of neurologic function in EAE models. However, in addition to its direct neuroprotective effect on axons, Craner et al. (86) demonstrated that Na channel blockers can also reduce neuroinflammation through its action on microglia and macrophages in EAE and MS. It was shown that there is robust increase of Na channel Nav 1.6 expression in activated microglia and macrophages in EAE and MS and that Na channel blockers phenytoin and tetrodotoxin can profoundly reduce inflammatory infiltrate and microglial phagocytic activity. This suggests that in addition to its direct neuroprotective effect, Na channel blockers may have worked in curtailing axonal degeneration because of its anti-inflammatory effect as well.

Although the scenario presented here is still hypothetical, current data on neuroinflammatory intermediates such as NO, potential mitochondrial dysfunction, hypoxic/ischemic pathological features in MS lesions (44), and reports of beneficial effects in EAE of neuroprotectants selected for study, based on models of anoxia/ischemia, all point to an interesting overlap in the mechanisms of axonal degeneration in seemingly disparate disorders such as ischemia, trauma, and neuroinflammatory diseases (76). However, unlike stroke and neurotrauma where the window of opportunity for treatment is so limited, the chronic relapsing–remitting course of MS gives us ample opportunity to intervene in the cascade and prevent widespread axonal damage via this mechanism before axonal injury accrues.

STRATEGIES FOR AXONAL PROTECTION

Axonal loss has been elegantly demonstrated in recent studies and believed to be the underlying process that determines disability. Thus, therapeutic strategies aimed at preventing neuronal damage might be the key toward preventing permanent disabil-
Despite the emerging mechanisms discussed before, the precise mechanism leading to axonal degeneration remains unclear. Most histopathologic data just give a snapshot of the disease process and makes a causal relationship difficult. However, the prevailing concept is that axons are injured during inflammatory demyelination. Alternatively, axonal injury is a consequence of loss of trophic support from myelin sheath. Most neuroprotective strategies are based on these premises. Emerging mechanisms have provided novel perspective on providing axonal protection.

Anti-inflammatory Strategies

Postmortem and biopsy studies of MS lesions suggest that axon loss is correlated with the magnitude of inflammation (1,9). Many believe that this relationship could be causal and that the axons are innocent bystanders in the surrounding inflammatory milieu during active demyelination. The clinical observation that the opographic pattern of irreversible, progressive neurological deficits in MS depends on the localization of the previous inflammatory attacks seems to favor this interpretation (87). Inflammatory substances like nitric oxide, glutamate, reactive oxygen species, and cytokines, such as tumor necrosis factor-α, are released during inflammatory episodes and are known to injure both axons and oligodendroglia. Modulation of inflammation at different levels might obviously be neuroprotective. Detailed discussion of different immunomodulatory agents are discussed elsewhere in this book. These agents are only effective during the RR phase when inflammation dominates the picture. It is well established that axonal loss during this phase can be substantial, therefore from the therapeutic point of view, early treatment with these agents is beneficial.

Remyelination Strategies

Myelin contributes to the structural and functional integrity of the axon. Strategies that aid in remyelination can confer axonal protection and can therefore be considered neuroprotective (88). Remyelination has been shown to be a common phenomenon in MS (89). However, this process is not robust enough to promote a functional and stable recovery of the myelin architecture. To improve myelin repair, several strategies are being explored. In principle, myelin repair can be achieved by promoting endogenous repair mechanisms, by providing an exogenous source of myelinating cells by transplantation and limiting damage to myelinating cells. The latter is usually done by immunomodulators or immunosuppressors that prevent further demyelination and are currently available. Repair of myelin lesion via the first two mechanisms are still under intense investigation and are yet to be proven to induce repair of MS lesions (90).

Promotion of Endogenous Remyelination

Our knowledge of myelin biology and oligodendrocyte development has exploded in recent years. As mentioned, some degree of remyelination has been show to occur in MS. Oligodendrocyte precursor cells (OPC) can be found in MS lesions (91,92). Hence one logical approach to repair MS lesions would be to induce inherent remyelination and promote regeneration. Administration of myelin-associated growth factors and recently, intravenous immunoglobulins (93) are part of this strategy. Nerve growth factor has been shown to delay the onset of clinical EAE and pathologically
prevented the full development of EAE lesions (94). Another neurotrophic cytokine, leukemia inhibitory factor prevents oligodendrocyte death in animal models (95). Fibroblast growth factor II gene therapy significantly reverts the clinicopathological signs of EAE (96) while platelet-derived growth factor enhances remyelination and reduces axonal abnormalities after toxic demyelination (97). CNTF, a neuropoietic cytokine, has been shown to protect oligodendrocytes from TNF-mediated cell death (98). However, studies of insulin growth factor (IGF-1) demonstrate how treatment at various stages and models of EAE may result in different effects. IGF-1 has been reported to reduce the clinical deficit and lesion severity in EAE (99). However, this effect was only transient and neither amelioration of clinical deficit nor remyelination was noted in the chronic phase (100). In addition, attempts to elevate levels of IGF-1 mRNA expression does not significantly change the extent of oligodendrocyte remyelination (101). The apparently contradictory reports underscore the complexity inherent in enhancing the gliogenic milieu of the CNS. Administration of a single growth factor is not expected to sustain a stable and long lasting remyelination. The proliferation, migration, and differentiation of progenitor cells into mature, myelinating oligodendroglial cells require a precisely timed sequence of growth signals that, in the case of MS patients, must be delivered to multiple lesions disseminated in space and time, inherently differing in their states of demyelination and remyelination (102). Furthermore, the success of such a strategy depends on the availability of an endogenous pool of progenitor cells ready to be induced to divide, migrate, and mature into functional myelinating oligodendrocytes. Finally, the initial insult that causes the demyelination in the first place has to be controlled lest the remyelinating cells be injured again.

**Transplantation**

An alternative remyelination strategy is to provide the MS brains with cells that would later develop into myelin forming cells, repopulate the disease regions of the CNS, and remyelinate the naked axons. There are numerous cell sources that can be transplanted. Oligodendrocytes at various stages of development have been tried successfully to achieve remyelination in several animal models (103). Schwann cells have also been shown to remyelinate the CNS (104) and offers the advantage of being accessible (e.g., from sural nerve biopsy) (105) and autologous, and therefore would not require immunosuppression. The olfactory ensheathing cells are good candidates as well for similar reasons (106). Finally, neural and embryonic stem cells have been demonstrated to differentiate into oligodendrocytes and remyelinate axons in vivo (107).

Many of the caveats in promoting endogenous remyelination also apply to transplantation. The potential for both immune rejection and malignant transformation of transplanted cells in addition to the ethical problems and limitation of donors have to be dealt with as well.

**Interruption of the Secondary Injury Cascade in Axons**

In the hypoxia-like model of axonal injury, we mentioned how different cellular insults result in impairment of energy production, which leads to a reversal of the Na–Ca exchanger. The resulting surge in the levels of Ca in the intracellular compartment drives enzymatic processes, which leads to cellular destruction. On the basis of these pathogeneses, treatment with Na channel blockers and Na–Ca channel
blockers may prevent this Ca driven autolysis of neuron. In EAE models and in vitro studies, Na channel blockers like phenytoin (85,108) and flecainide (84) may have succeeded in providing axonal protection through this mechanism. Bepridil, an inhibitor of Na–Ca exchange, has been shown to protect axons from injury caused by NO in vitro (109). These drugs are worth looking into as some of them have already been in the market for decades. Once proven effective in MS, we have an immediate addition to our arsenal against MS that has a relatively well-established safety profile.

SURROGATE MARKERS OF AXONAL LOSS

In contrast to clinical outcome measures such as relapse and expanded disability status scale (EDSS), which are insensitive and poor at reflecting disease activity, the objective, sensitive, and quantitative changes measured by MRI provides an additional tool for prognosticating disease course and measuring the outcome of new therapies in MS. Several MRI techniques are now available with reasonable specificity for axonal damage. Here, we focus on magnetic resonance (MR) measures of N-acetyl aspartase (NAA) level, T1 hypointense lesion, and brain atrophy. We also look at the utility of these markers as outcome measures in clinical trials. However, as discussed below, it should be noted that use of data from these MRI metrics requires an appreciation of what is being measured and the potential errors and difficulties. Clearly, none of these measures meets the stringent criteria for a validated surrogate in MS. However, the changes detected by these techniques reflect an underlying pathologic process that is most likely related to disease activity and clinical progression. Therefore, in a complex disease with a high degree of longitudinal variability of clinical signs and symptoms within and between patients, these non-conventional MRI techniques provide a promising tool to noninvasively study the pathological substrate of disability, predict disease progression, and the effect of treatment on an important aspect of MS pathology.

N-acetyl Aspartase

NAA is an abundant free amino acid present in the vertebrate brain and is enriched only in neurons and its processes (110). This neuronal specificity makes it an ideal marker for monitoring neuronal and axonal health. In acute stages of MS, reduced NAA is partly reversible, restricted to lesion areas, and correlates with reversible functional impairment (111–114). In chronic stages of the disease, reduced NAA is also detected in NAWM, suggesting axonal damage or Wallerian degeneration outside MS lesions (115–117). In addition, many studies support the correlation of NAA levels with disability overtime (111,116,118) and with executive function in MS (21).

Decreased NAA in MS was initially interpreted as a result of irreversible axonal loss. However, the observation that NAA in acute MS lesions is reversible to some extent indicated that NAA levels also reflect reversible axonal dysfunction. The function of NAA is unknown, although participation in protein synthesis, osmotic regulation, and metabolism of neurotransmitters such as aspartate and N-acetyl-glutamate has been suggested (119–122). After synthesis in mitochondria from L-aspartate and acetyl-CoA, NAA is transported to the neuronal cytoplasm where it is present in high concentrations (123,124). It has recently been suggested that neuronal NAA is released into the extracellular space, and subsequently taken up
and degraded by oligodendrocytes (125). Myelin or myelin forming cells can dynamically influence various axonal properties such as the distribution of axolemmal ion channels (126–128), phosphorylation or dephosphorylation of neurofilaments (46), and axon caliber (129,130). Analogous, it is possible that inflammatory demyelination and remyelination may dynamically influence the activity of axonal enzymes involved in NAA metabolism, hereby transiently affecting NAA levels. In addition, it is possible that NAA metabolism is related to neuronal activity in a tract. For example, acute deafferentation in the CNS causes trans-synaptic decreases of NAA levels without ultrastructural abnormalities, indicating that impaired function reduces neuronal NAA (131). Reduced levels of NAA might therefore reflect a number of mechanisms, such as reversible neuronal/axonal damage due to inflammatory demyelination, altered neuronal/axonal metabolism, changes in neuronal activity, or axonal loss (111,114,117,131).

In vivo NAA can be reliably measured noninvasively by magnetic resonance spectroscopy (MRS)—one of the modern quantitative MR techniques that have the potential to overcome some of the limitations of conventional MRI (cMRI) in accurately assessing lesion burden in MS (Fig. 3). Other modern MR techniques like magnetization transfer and diffusion weighted MRI enable one to more specifically quantify the extent of structural changes occurring within and around the MS lesion (132). MRS can add information on the biochemical nature of such changes, with the potential to significantly improve our ability to monitor inflammatory demyelination and axonal injury. At present, the technique remains technically demanding and suffers from poor spatial resolution, but with future technical advances it may become more routine.

That MRS detection of NAA concentration is an accurate measure of axonal density has been confirmed by histology of biopsied samples (133). To determine NAA levels in MS spinal cords, high-pressure liquid chromatography (HPLC) analysis of whole cord cross sections was performed postmortem. At cervical and lumbar levels, average NAA levels were significantly decreased by 53% and 55%, respectively (134). Since these patients were severely disabled, the data indicates that reduced NAA levels in chronic MS, as detected by MRS, can reflect irreversible functional

![Figure 3](image.png)  
**Figure 3** Proton magnetic resonance spectra from a normal brain (A), normal-appearing white matter of a patient with multiple sclerosis (B), and chronic periventricular plaque of the same patient with multiple sclerosis. *Source:* From Ref. 178.
impairment. Moreover several studies also show that NAA levels are inversely correlated with disability status as measured by clinical indices like EDSS (118,134). A few studies, however, are reported with no correlation (135,136). This is not surprising, however, as clinical indices like the EDSS have been criticized for their failure to reflect the actual extent of disease pathology due to its weighing toward cerebellar and spinal cord deficits (137,138). In addition, the effect of lesion location and CNS plasticity that is known to occur in MS (139,140) makes the value of clinical indices in assessing the full burden of disease in MS less useful. In contrast, NAA dynamics yield a direct measure of the brain’s pathologic structure load without the distorting overlay of function, thereby more objectively predicting the course of organic pathology, which may be more appropriate for monitoring disease progression in clinical trials.

Falini et al. (141) tested the utility of MRS in defining the extent of metabolic changes in benign versus SPMS and found significant differences in NAA pattern according to the phase (acute vs. chronic) and the clinical form (benign vs. progressive) of the disease. Using whole brain NAA dynamics, Gonen et al. (135) was able to define subgroups among RRMS patients based on the rate of decline of NAA levels that may help stratify patients for active therapeutic intervention. This is particularly compelling now, in the light of the observation that axonal injury starts early in the course of the disease and that partially effective treatment for MS is available for certain group of patients.

To assess treatment effects on axonal injury, several clinical trials utilized NAA level as an outcome parameter. Interferon beta (IFNβ) has been shown to provide some benefit in MS patients. However, the mechanisms of action of this drug are incompletely understood and effects of IFNβ on axonal injury are not known. One small study examining the effects of IFNβ-la in patients with RRMS showed that once weekly IFNβ-la do not change the levels of NAA (142). Another pilot study tests the effects of IFNβ-lb and reports a higher NAA levels in the treatment group compared with controls after a year. This suggests that patients with MS suffer from chronic sublethal injury that is at least partially reversible with IFNβ-lb treatment (143). Subsequent study, however, shows that this result cannot be generalized as NAA levels continue to drop in both treatment and control group suggesting that IFNβ l-b does not always reverse or arrest progression of axonal injury in patients with MS (144).

**T1 Hypointensity**

The diagnostic hallmark of MS is hyperintense lesions on T2-weighted MRI scans (145). Despite high sensitivity to tissue change, these T2 white matter signal abnormalities are pathologically nonspecific and are of limited value in assessing disease progression and therapeutic response. T2-weighted MRI is collectively sensitive to a variety of pathological processes, such as inflammation, edema, demyelination, axonal loss, and repair processes. All of these processes may change the T2 signal in a similar way (146). This has led to difficulties in assessing actual burden of disease in MS and in correlating disability, which is determined mainly by axonal loss.

Chronic T1 hypointense lesions (also known as black holes) are defined as lesions that have lower signal intensity than the surrounding white matter, typically with signal intensity equal to or lower than grey matter (147). The prevalent view is that T1 lesions represent a more severely damaged subset of MS-induced lesions. Histopathologic analyses revealed that chronic T1 hypointense lesions primarily represent extensive tissue destruction, failure of remission, and axonal loss (148).
and the return to T1 isointensity has been proposed as an indicator for remyelination (149). This makes T1 hypointensity a more specific surrogate of axonal injury than T2 lesions. There has been considerable interest therefore, to see the relationship between T1 lesions and clinical outcomes, including disability measures. Correlations between changes in T1-weighted lesion load and disability, as assessed by EDSS, have been demonstrated in several studies in RRMS and SPMS. In a study correlating changes in hypointense lesion load on T1-weighted spin-echo MR images with changes of disability in MS, 46 patients with clinically definite MS were followed-up for 40 months. A significant correlation between baseline disability and hypointense lesion load [Spearman rank correlation coefficient (SRCC) = 0.46, \( P = 0.001 \)] was demonstrated. In secondary progressive patients, the rate of accumulation of these “black holes” was significantly related to progression rate (SRCC = 0.81, \( P < 0.0001 \)) (150). In addition, a study in 15 patients with MS and varying levels of disability demonstrated a strong correlation between T1 lesion load and EDSS scores (\( r = 0.71 \)). Moreover, patients with RRMS have a lower T1/T2 ratio than those with SPMS. Studies have shown that the T1/T2 ratio increases over time, especially in patients with SPMS (16), indicative of progressing demyelination, axonal loss, or both as the disease develops. Axonal loss generally becomes more severe during the course of the disease or as repair mechanisms become exhausted, resulting in a greater number of black holes.

A study of 68 RRMS patients investigated whether subcutaneous IFN\( \beta \)-la modifies the course of new MS lesions. The course of new Gd-enhancing lesions were followed during a six months observation and treatment. In the six months pretreatment period, significantly more new enhancing lesion developed into T1 black holes than during active treatment (49 vs. 15%; \( P = 0.001 \)) (151). Another study evaluated the effect of weekly treatment with intramuscular IFN\( \beta \)-la (Avonex\textsuperscript{TM}) in patients with RRMS in reducing the rate of increase in T1 hypointense lesions volume relative to placebo. In placebo patients there was a 29.2% increase in the mean volume of T1 hypointense lesions (median 124.5 mm\(^3\)) over two years (\( P < 0.001 \) for change from baseline), as compared to an 11.8% increase (median 40 mm\(^3\)) in the IFN\( \beta \)-la-treated patients (change from baseline not significant) (152). These treatment group comparisons, however, did not reach statistical significance.

Another study evaluated whether glatiramer acetate (GA) is able to favorably modify the evolution of new MS lesions by reducing the proportions of lesions that develop into permanent black holes. Almost 239 patients with MS enrolled in a placebo-controlled trial were monitored monthly with cerebral MRI. The percentage of new lesions that evolved into T1 hypointense lesions was lower in GA-treated than in placebo patients on scans at seven (18.9% and 26.3%; \( P = 0.04 \)) and eight (15.6% and 31.4%; \( P = 0.002 \)) months after lesion appearance. This indicates that GA may exert a beneficial effect on the events leading to irreversible axonal disruption once lesions are formed (153).

**Tissue Atrophy**

CNS atrophy reflects the net result of irreversible and destructive pathological processes in MS. Axonal damage and loss, chronic demyelination, and gliosis contribute to a reduction in brain parenchymal tissue volume and a corresponding expansion of cerebrospinal fluid (CSF) spaces. A number of reports indicate that disease progression in MS is reflected by volume loss of CNS tissue (154–157). Atrophy is thus a useful surrogate marker for monitoring disease progression and the efficiency of
MS therapeutics. The most commonly used surrogate marker for disease progression is total brain lesion volume as measured on T2 weighted MRI scans. As mentioned, this measurement has relatively low pathological specificity and its correlation to performance is poor (157,158). MRI studies of atrophy, however, have demonstrated a correlation between clinical disability and atrophy of cerebellum (154), spinal cord (155), and cerebral tissue (156). Reliable methods of measuring the rate of tissue atrophy in MS from early stages of the disease could, therefore, be useful for the monitoring of MS patients.

The spinal cord, frequently affected in MS patients (159), is considered a suitable model to study the relation between atrophy and clinical progression due to the impact of motor disability on EDSS (157). Spinal cord atrophy as determined by MRI, but not total brain lesion load, correlates with clinical disability in MS (155,160,161). In spinal cords, from chronic MS patients with severe disability (EDSS > 7.5) and significant axonal loss in spinal cord lesions (see above), average cervical spinal cord cross section area was reduced by 25% (162). The amount of cervical cord atrophy in a comparable patient subgroup investigated by MRI was 28% (155). These data suggest that axonal loss contributes to spinal cord atrophy in MS.

In the brain, the periventricular white matter is frequently affected by MS lesions, which might contribute to the progressive enlargement of the lateral ventricles often observed in MS patients (Fig. 4) (13,37,163). In a serial MRI study, progressive cerebral atrophy as determined by the volume calculated from four central brain slices was significantly more pronounced in patients with worsening disability indicating axonal damage or degeneration (156). Interestingly, progressive brain atrophy, as seen by MRI, has also been reported in RRMS patients with short disease duration. During the two years of observation, brain atrophy in RRMS patients with mild to moderate disability increased yearly in many cases without clinical manifestations (36,37). On the same population of relapsing patients, a new sensitive measure of whole-brain atrophy was applied (36). The brain parenchymal

![Figure 4](image-url) Progressive brain atrophy during the course of multiple sclerosis. Magnetic resonance images from a control subject without disease (male, age 31) (A); a patient with relapsing–remitting multiple sclerosis (female, age 36) with disease duration of two years (B); and a secondary progressive multiple sclerosis patient (female, age 43) with disease duration of 19 years (C). As shown in (B) and (C), brain tissue volume decreases and ventricular volume increases with disease severity. Demyelination and axonal loss contribute to tissue loss. Source: From Ref. 23.
fraction (BPF), defined as the ratio of brain parenchyma to the total volume within the brain surface contour, was highly reproducible thus allowing precise comparison of individual brain volumes from year to year. The BPF declined at a highly significant rate during each of two years of follow-up in these patients and was significantly reduced compared with age- and sex-matched control individuals.

Unlike white matter, the contribution of cortical pathology in MS has not been fully appreciated until recently. There is increasing evidence that cortical grey matter is involved in the disease process (2,164), but it is not known precisely how the cortex is affected, or what regions are predominantly involved. A study in RRMS and primary progressive (PP) MS patients using T1-weighted images to estimate cortical thickness demonstrated significant reductions in cortical volume relative to normal control subjects. In patients with MS, there was a significant correlation between EDSS score and cortical volume, which was stronger in the patients with PPMS (165). Sailer et al. (166) reported that patients with MS had a significantly reduced mean overall thickness of the cortical ribbon relative to the control group, while there were significant correlations with disability, disease duration, and T1 and T2 lesion volumes. In addition, they observed focal thinning in the motor cortex region in patients with long-standing disease or severe disability, and there was also focal thinning in distinct cortical regions (frontal and temporal) in patients with mild disability and those who were in the early stages of the disease. Measures of cortical atrophy may well provide additional information on disease pathology, and in the future may serve as a prospective marker of disease progression.

Although demyelination and reduced axon diameter may decrease CNS tissue volume, axonal loss from the onset of disease is a plausible contributor to atrophy in MS for the reasons discussed above (36,37,157,163). However, as with other surrogate MRI metrics, caution should be exercised when extrapolating conclusions from these data. For example, the lack of correlation between axonal loss and atrophy in some spinal cord lesions of chronic MS patients (134), and the prominent upregulation of glial fibrillary acidic protein observed in many chronic MS lesions, suggests that other factors such as the extent and nature of compensatory astrogliosis can influence tissue volume in MS. Furthermore, measures of atrophy may also contain element of transient volume changes (e.g. edema, corticosteroid-induced tissue shrinking), which may obscure the true amount and rate of tissue degeneration. For example, a large edematous lesion may increase the apparent brain parenchymal volume and thus paradoxically, MS brain treated with agents effectively controlling CNS inflammation may appear more atrophic than an inflamed one in the short term. This is especially a problem in the RR phase of MS where inflammation is a dominant picture than in the progressive phase. Axonal loss is expected to contribute to atrophy while inflammation tend to increase tissue volume. This should all be taken into consideration when judging the validity of the result of clinical trials where atrophy is an outcome measure.

Rudick et al. (36) report a reduction of brain atrophy progression in a two-year clinical trial of IFNβ-la, but this is apparent only during the second year of the trial. This is despite a remarkable decrease relapse rate and Gd-enhancing lesion. This probably indicates a dissociated effect of treatment on inflammation and axonal loss. Alternatively, the effect of treatment on measures of atrophy may be delayed as Wallerian degeneration, which contributes to atrophy, can take months to years in mammals (167). In the three-year European trial of IFNβ-lb in SPMS, no significant difference was observed between placebo and treatment groups in the rate of brain atrophy (168). Interestingly, an exploratory subgroup analysis of patients with and without Gd-enhancement showed that patients with active inflammation at baseline have a
higher rate of brain atrophy with IFNβ-lb than placebo. As discussed above, these data emphasize the difficulty in segregating the anti-inflammatory activity (which reduce brain volume) and the anti-atrophy effects of IFN making interpretation difficult.

Another study examined the effects of GA on brain atrophy in patients with RRMS over a nine-month double blind, placebo controlled phase, and a nine-month open-label phase. In this short study, treatment also failed to arrest the reduction in brain volume compared with placebo (169).

Overall, the available information to date indicates that preventing axonal loss as measured by this surrogate, medical treatment seems to be far more difficult than preventing new focal activity. This might indicate that these two phenomena are temporally disconnected or are caused by different processes (147).

CLINICAL IMPLICATIONS

What are the consequences of acute, chronic, and cumulative axonal loss in the clinical progression of MS? The evolving concept that MS is an inflammatory neurodegenerative disease provides a hypothetical framework that explains disease progression and development of permanent neurological disability in affected patients (Fig. 5). In this model, axonal degeneration begins at disease onset (1), which may not necessarily mean the first documented neurologic dysfunction. Given the extensive redundancy and remarkable plasticity of the brain, it is conceivable that significant axonal injury began well prior to the first neurologic attack. About 50% to 70% of those with clinically isolated syndrome, suggestive of MS, have evidence of old brain lesions on unenhanced MRI reflecting a more advanced burden of disease in MS (170). Also, atrophy can be observed in patients with the first presenting signs indicative of MS. Measures of atrophy suggest that axonal loss occurs well before the development of clinical deficit (171). Lesions detected by MRI outnumber clinical relapses by as much as 10:1 (172).

Typically, MS in young patients tend to start as RR, which is characterized by inflammatory attacks, reversible neurologic dysfunction, or residual deficit with
variable periods of remission. This is a highly variable phase in terms of symptomatology and duration. Clinical studies have shown that the time from clinical onset of MS to an EDSS score of 4 (usual threshold of irreversible disability) range from 1 to 33 years (173,174). During this phase, the underlying mechanism for relapse appears to be the recurrent inflammatory demyelination leading to functional or structural axonal impairment which is responsive to immunomodulatory therapy. In addition, MRI studies have shown that measures of the active inflammatory component of MS such as contrast enhancing and T2 lesions correlates well with disease course in this phase (171). The need for an early and continuous treatment at this stage, despite paucity of residual deficit, cannot be overemphasized as axonal damage has been suggested to be maximal early in the disease process and decreases over time (175).

After a decade, 50% of RRMS patients will have converted to the SP form of MS. After 20 to 25 years, 90% of patients will have become progressively disabled (176) with a major disability of lower extremity function and decline in ambulation in most patients. In contrast to the RRMS phase, MRI studies that reflect the neurodegenerative aspect of MS like brain atrophy (171) and NAA levels (134) are the only ones that have a good correlation with disability. Hence, immunomodulatory treatments seem to work before fail to exert the same influence once the progressive phase has set in. Furthermore, once irreversible disability is reached and SPMS ensues, the time course for EDSS score from 4 to 7 is similar in most patients and is not affected by the presence or absence of relapse (before or after the progressive phase) (173,174).

The sharp contrast in clinical and pathologic behavior of MS in these two stages suggests that different mechanisms cause axonal loss at different stage of the disease. Inflammatory axonal transection and lack of myelin trophic support may be responsible for axonal loss and subsequent disability in the initial stage of the disease. In the progressive phase, the pathogenesis for neuroaxonal loss is poorly understood. The specific role inflammation plays in disease progression is not well-defined. Corollary to this, even if inflammation and relapse are effectively suppressed, progression of axonal loss and subsequent disability remains unaltered, which led many to embrace the interesting possibility that MS is a primary neurodegenerative disease modified by superimposed episodes of focal inflammation.

A recent study was conducted (177) to investigate the relationship between age and rate of disability progression in a large hospital-based cohort of MS patients. Analysis showed that disease duration being equal, older age correlates with faster decline in neurologic function. EDSS severity is inversely correlated with age of onset and is positively correlated with current age. Patients with early onset tend to approach the same level of disability of those with late onset when they reach a similar age.

The data presented here support the concept that MS is an inflammatory neurodegenerative disease characterized by progressive disability with the rate of disability accelerating with increasing age. However, unlike other neurodegenerative conditions where subclinical neuronal loss is the rule, the RR period of MS provides a huge window of opportunity to identify patients and pretreat individuals before significant neuronal loss and disability accrues. In this respect, there is a good reason for optimism in MS, which is certainly not the case a decade ago.

CONCLUSION

On the basis of current evidence, MS can be considered an inflammatory neurodegenerative disease. The role of axonal injury in determining permanent neurologic
disability is well-founded and probably holds the key to the ultimate goal in MS—that of prevention and recovery from disability. In recognition of this, mechanism of axonal injury and protection has been given due attention in recent years. Our understanding of these mechanisms has been significant yet their contribution, if at all they exist, in vivo in humans remains to be determined. Current knowledge indicates that there are diverse targets for intervention during the various phase of the disease that can be translated to treatment.

The predominantly inflammatory picture of the initial phase of the disease makes immunomodulators an effective treatment in controlling the recurrent episodes of inflammatory demyelination known clinically as relapse. At present, there are a number of drugs with documented effect during RRMS, for example IFNβ and GA. Given the role of persistent inflammation as a cause of axonal injury, even during clinically silent stages of RRMS, early aggressive anti-inflammatory treatment might, therefore, also have indirect neuroprotective effects. In addition, emerging pathogenetic mechanisms of axonal injury in MS should be vigorously pursued as they can provide additional neuroprotective avenues.

Satisfactory therapy for the progressive form of MS is currently lacking. From the perspective of inflammation the lesions in this phase are relatively silent, but axonal loss continues and is reflected clinically by the patient’s progressive deterioration. Unfortunately as has been pointed out, as to what mechanisms the axonal injury operate during this period is still largely unknown. On the basis of the recent evidences, inflammation is not one of them.

A lot of drugs for MS are currently in various stages of development. It is clear that for therapy to be successful in preventing or delaying disability, their effects on axonal damage should be investigated. As evidence mounts that MS consists of distinct subforms, the choice of treatment for individual patients should ideally be determined by knowledge of the specific underlying pathophysiological mechanisms and profiles of the available drugs. Although strides have been made, better understanding of the pathogenetic mechanism of axonal injury in MS may lead to more efficient therapeutic strategies for the neurodegenerative aspect of MS.

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Activities of daily living (ADL), 282
Acute disseminated encephalomyelitis (ADEM), 67, 69, 73
pathology of, 127
Acute treatment, 301
ADEM. See Acute disseminated encephalomyelitis.
ADL. See Activities of daily living.
Adrenocorticotrophic hormone (ACTH), 304
Adult-onset multiple sclerosis (AOMS), 23
Age of onset, 158
Alemtuzumab, 395
ALS. See Amyotrophic lateral sclerosis.
Altered peptide ligand (APL), 409–411
Alzheimer’s disease (AD), 50, 54–56
Amantadine (Symmetrel), 283
Amyloid precursor protein (APP), 478
Amyotrophic lateral sclerosis (ALS), 5
Animal infectious agents, 82–83
Ankle foot orthosis (AFO), 288
Antecedent infections, 16
Anti-adhesion molecule antibodies, 386–387, 391
Anti-B-cell antibodies, 397
Antibodies
anti-adhesion molecule, 386–387, 391
anti-B-cell, 397
anti-cytokine, 392
anti-T-cell, 395–396
pathogenic, 459
reparative, 459–460
Antibodies, monoclonal, 385, 386
Antibody-mediated CNS repair, mechanism of, 464–466
Anticholinergic medication, 274
Anti-cytokine antibodies, 391–392
Antigen, universal, 362
Anti-inflammatory strategies, 487
Anti-T-cell receptor antibodies, 395, 396
Anti-thymocyte globulin (ATG), 431
APL. See Altered peptide ligand.
Apolipoprotein E (APO-E), 233
Apoptosis markers, 47, 234
APP. See Amyloid precursor protein.
Apparent diffusion coefficient (ADC), 183
Aricept, 293
Assistive technologies (ATs), 295–296
Ataxia, 291, 292
Autoimmune diseases, 67
Autoimmune hypothesis, 103–104
Autoimmune responses, alteration of, 412–413
Autologous stem cell transplantation, 426–427
Autonomic disturbance, 166
Axon
damage, 321, 322
degeneration, 323
hypothesis, 201
injury, 481–482, 485, 486
injury markers, 232, 489
loss, 378, 480, 489
pathology, 135, 136, 477
protection, strategies for, 486–487
Axonal injury mitochondrial component of, 485–486
Baclofen pump, 273, 287
Balo concentric sclerosis (BCS), 139, 140
B-cell-specific transcription factor, 16
BCS. See Balo concentric sclerosis.
Biomarkers, potential, 225
cytokines, 226, 227
tumor necrosis factor (TNFα), 227
Black holes, 358
Blood oxygenation level dependent (BOLD), 183
Body cooling, 289, 291
Body fluids, 226
Botox, 273, 287
Botulinum toxin (Botox), 273, 287
Bowel dysfunction, 274–275
Bracing, 289
Brain atrophy, 324
Brain parenchymal fraction (BPF), 493–494
Brain stem symptoms, 160–161
Brainstem auditory evoked potentials (BAEPs), 244, 247, 248

Canine distemper virus (CDV), 69, 83–85
Carbamazepine, 276
Cardiotoxicity, 377
CDV. See Canine distemper virus.
Cell death, induction of, 380–381
Cell subpopulations, 230
Cell transplantation, 457
Cerebellar manifestations, 162–163
CHAMPS. See Controlled High Risk Subjects Avonex Multiple Sclerosis Prevention Study.
Charcot-Marie-tooth neuropathy type 1 (CMT1), 483
Chlamydia pneumoniae, 17, 81–82
Choroid plexus cells, 79
Chronic inflammatory demyelinating polyneuropathy (CIDP), 67, 69
Ciliary neurotrophic factor (CNTF), 482
Clinically definite multiple sclerosis (CDMS), 305, 318
Clonazapam (Klonopin), 276
Cognitive impairment, 293–294
Combination therapy, 443–445
Complementarity-determining region (CDR), 28
Controlled High Risk Subjects Avonex Multiple Sclerosis Prevention Study (CHAMPS), 319
Cooling, of body, 289, 291
Corticosteroids, 304
Costimulatory molecules, 228–229
CTX. See Cyclophosphamide.
C–reactive protein (CRP), 380
Cyclophosphamide (CTX), 311, 312, 424, 426
Cylert, 283
Cytokine network, 381
Cytopathic effect (CPE), 343

Dantrium, 273
Dantrolene (Dantrium), 273
Deep brain thalamic stimulation (DBS), 291

Demyelinating activity, 116, 118
definition of, 116
Demyelination, 69, 453
persistent infection, 70–71
transient infection or “hit-and-run” hypothesis, 71–73
Depression, 277
Devic’s syndrome, 49
Diet, effects on disease, 14
Diplopia, 277
Disease modifying drugs (DMD), 425
Dual-echo imaging, 181
Dysarthria, 161
Dyssynergia, 274

Earlier onset multiple sclerosis (EOMS), 23
EBV. See Epstein–Barr virus.
EBV nuclear antigen (EBNA), 77
Eichhorst’s description, 41
ELISA. See Enzyme-linked immunosorbent assay.
Embryonic stem (ES) cells, 458
Encephalitogenic peptides, 44
Encephalomyelitis, perivenous, 140–141
Endogenous remyelination, promotion of, 487, 488
Endophenotypes, 50
Enzyme-linked immunosorbent assay (ELISA), 81, 83, 534, 539
EOMS. See Earlier onset multiple sclerosis.
EP testing multimodality, 253
ECS. See Evoked potentials.
Epstein–Barr virus (EBV), 17, 75–78
ETOMS-CHAMPS, 324, 426
European intravenous immunoglobulin in secondary progressive multiple sclerosis (ESIMS), 307
Event-related potentials (ERP), 244, 253
Evidence-based medicine (EBM), 336, 337
Evoked potentials (ECS), 243, 252, 256
Expanded disability status scale (EDSS), 50
Experimental autoimmune encephalomyelitis (EAE), 13, 104

Fast-fluid-attenuated inversion recovery (FLAIR) scans, 185
Fatigue, 164, 271, 282, 283
Fitness, 294, 295
FLAIR. See Fast-fluid-attenuated inversion recovery.
Index

GA. See Glatiramer acetate.

GAMES. See Genetic Analysis of Multiple Sclerosis in Europeans.

Gamma activation sequence (GAS), 334

Genetic Analysis of Multiple Sclerosis in Europeans (GAMES), 46–49

Gitter cells, 116

Glatiramer acetate (GA), 351, 415, 463 advantage of, 366 clinical trials, 351–356 effect of, 358, 443 immunological activity of, 361

Glial fibrillary acidic protein (GFAP), 233

Gliosis markers, 232–233

Gray matter (GM), 180 damage, assessment of, 199–200 pathology, 136–137

Guillain-Barre syndrome (GBS), 67, 69, 81

Gustation, 182

Haplotype spanning, 45

Hematopoietic stem cells (HSC), 427

Hematopoietic stem cell transplantation (HSCT), 427

Hepatitis B vaccination, 18

Hereditary motor and sensory neuropathy (HMSN), 253

Herpesvirus 1, 18

Herpesvirus 6 and 7, 17–18


High-dose methylprednisolone (HDMP), 302

HLA. See Human leukocyte antigen.

Human antihuman antibody response (HAHA), 386

Human antimouse antibody response (HAMA), 386

Human brain microvascular endothelial cell (HB-MVEC), 335

Human endogenous retrovirus (HERV), 19

Human herpesvirus 6 (HHV-6), 17, 18, 71, 79, 81

Human infectious agents

EBV hypothesis, 76

measles virus, 73–74

Human leukocyte antigen (HLA), 43, 85

Human retrovirus elements (HERVs), 78, 79

Humoral immunity, 101–102

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors, 413

Hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene, 100

Hypoxia inducible factor (HIF)-1α, 129

Idiopathic inflammatory demyelinating disease, 49

IFN. See Interferon.

Immune regulation, restoration of, 363

Immunoglobulins, 229

Inderal, 276

Inducible nitric oxide synthase (iNOS), 336

Infections, antecedent, 16

Influenza virus, 16–17

Interferon (IFN), 333

methotrexate, 447–448 methylprednisolone, 447–448 therapeutic effects of, 445

Interferon beta (IFN-β), 134, 333 effects of, 335, 336, 340 efficacy of, 339 forms of, 334

Interferon regulatory factors (IRFs), 334

Interferon stimulated response element (ISRE), 333

Intermediate phenotypes, 50

Intravenous immunoglobulin (IVIg), 302, 306–308, 326

Klonopin, 276

Latency period, 13

Left ventricular ejection fraction (LVEF), 377

Leukemia, 379

Linkage disequilibrium (LD), 43, 46, 49, 58

Lovastatin, 413

MAG. See Myelin associated glycoprotein.

Major myelin proteins (MMP), 116

Magnetic resonance image (MRI) techniques, aspects of, 182, 183

Matrix metalloproteinases (MMPs), 96, 230, 335

MBP. See Myelin basic protein.

Mesenchymal stem cells (MSC), 435

Metamucil, use of, 275

Methotrexate, 447

Methylprednisolone, 448–449

Mitoxantrone (MTX), 310–311, 448–449 clinical studies, 373, 374

Human brain microvascular endothelial cell (HB-MVEC), 335

Human endogenous retrovirus (HERV), 19

Human herpesvirus 6 (HHV-6), 17, 18, 71, 79, 81

Human infectious agents

EBV hypothesis, 76

measles virus, 73–74

Human leukocyte antigen (HLA), 43, 85

Human retrovirus elements (HERVs), 78, 79

Humoral immunity, 101–102

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors, 413

Hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene, 100

Hypoxia inducible factor (HIF)-1α, 129

Idiopathic inflammatory demyelinating disease, 49

IFN. See Interferon.

Immune regulation, restoration of, 363

Immunoglobulins, 229

Inderal, 276

Inducible nitric oxide synthase (iNOS), 336

Infections, antecedent, 16

Influenza virus, 16–17

Interferon (IFN), 333

methotrexate, 447–448 methylprednisolone, 447–448 therapeutic effects of, 445

Interferon beta (IFN-β), 134, 333 effects of, 335, 336, 340 efficacy of, 339 forms of, 334

Interferon regulatory factors (IRFs), 334

Interferon stimulated response element (ISRE), 333

Intermediate phenotypes, 50

Intravenous immunoglobulin (IVIg), 302, 306–308, 326

Klonopin, 276

Latency period, 13

Left ventricular ejection fraction (LVEF), 377

Leukemia, 379

Linkage disequilibrium (LD), 43, 46, 49, 58

Lovastatin, 413

MAG. See Myelin associated glycoprotein.

Major myelin proteins (MMP), 116

Magnetic resonance image (MRI) techniques, aspects of, 182, 183

Matrix metalloproteinases (MMPs), 96, 230, 335

MBP. See Myelin basic protein.

Mesenchymal stem cells (MSC), 435

Metamucil, use of, 275

Methotrexate, 447

Methylprednisolone, 448–449

Mitoxantrone (MTX), 310–311, 448–449 clinical studies, 373, 374

Hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene, 100

Hypoxia inducible factor (HIF)-1α, 129

Idiopathic inflammatory demyelinating disease, 49

IFN. See Interferon.

Immune regulation, restoration of, 363

Immunoglobulins, 229

Inderal, 276

Inducible nitric oxide synthase (iNOS), 336

Infections, antecedent, 16

Influenza virus, 16–17

Interferon (IFN), 333

methotrexate, 447–448 methylprednisolone, 447–448 therapeutic effects of, 445

Interferon beta (IFN-β), 134, 333 effects of, 335, 336, 340 efficacy of, 339 forms of, 334

Interferon regulatory factors (IRFs), 334

Interferon stimulated response element (ISRE), 333

Intermediate phenotypes, 50

Intravenous immunoglobulin (IVIg), 302, 306–308, 326

Klonopin, 276

Latency period, 13

Left ventricular ejection fraction (LVEF), 377

Leukemia, 379

Linkage disequilibrium (LD), 43, 46, 49, 58

Lovastatin, 413

MAG. See Myelin associated glycoprotein.

Major myelin proteins (MMP), 116

Magnetic resonance image (MRI) techniques, aspects of, 182, 183

Matrix metalloproteinases (MMPs), 96, 230, 335

MBP. See Myelin basic protein.

Mesenchymal stem cells (MSC), 435

Metamucil, use of, 275

Methotrexate, 447

Methylprednisolone, 448–449

Mitoxantrone (MTX), 310–311, 448–449 clinical studies, 373, 374
Mitoxantrone (MTX)
- Mechanisms of action, 579
- Toxicities, 449

MMPs. See Matrix metalloproteinases.

Modafinil (Provigil\textsuperscript{1}), 283

MOG. See Myelin oligodendrocyte glycoprotein.

Mononuclear leukocyte infiltration, 101

Motor evoked potentials (MEPs), 252

Motor symptoms, 158–159

Motor-Uhthoff’s phenomenon, 288

MS-associated retrovirus (MSRV), 19

MTX. See Mitoxantrone.

Multiple sclerosis (MS), 1, 41
- Benign, 24
- Biomarkers, role, 223, 224
- Candidate agents in, 73
- Catastrophic, 302
- Clinical variability, prognosis, 22, 23
- Diagnosis of, 153
- Epidemiology, 67, 68
- Etiology, clues, 25, 28
- Familial factors and genetic susceptibility, 22, 23
- Lesions, types of, 118, 120
- Marburg, 139
- Pathophysiology, 194
- Prevalence, 6
- Tumefactive, 141

Murine hepatitis virus (MHV), 454

Myasthenia gravis (MG), 96

Myelin associated glycoprotein (MAG), 98, 116

Myelin basic protein (MBP), 72, 116, 231

Myelin components, 231, 232

Myelin oligodendrocyte glycoprotein (MOG), 98, 102, 116, 231, 459

Myelin proteins, minor, 116

Myelin reactive T cell, 99

Myelin-related axonal loss, 483

Mysoline\textsuperscript{1}, 276

N-acetyl aspartate (NAA), 489, 490

Natalizumab, 387, 390

NAWM. See Normal appearing white matter.

Nerve blocks, 287

Neural cell adhesion molecule (NCAM), 234

Neural stem cells, multipotential, 458

Neurofilament light chain (NFL), 232

Neuromuscular fatigue, 272

Neuromyelitis optica (NMO), 54

Neuronal pathology, 480

Neuroprotection, 363–364

NMO (Devic’ disease), 141, 142

NMO. See Neuromyelitis optica.

Nocturia, 274

Nonparametric linkage (NPL), 42

Normal appearing white matter (NAWM), 130, 137, 138, 179, 183, 194, 197, 199

Normal-appearing brain tissue (NABT), 193, 197, 199

OCBs. See Oligoclonal bands.

OCT. See Optical coherence tomography.

Olfaction, 162

Oligoclonal bands (OCBs), 54, 101–102, 229

Oligodendrocyte, 123, 124

Oligodendrocyte precursor cells (OPC), 487

ON. See Optic neuritis.

OPN. See Osteopontin.

Optic nerve damage, assessment of, 200, 201

Optic neuritis (ON), 4, 24, 25, 187

Optic neuritis, monosymptomatic, 49

Optic neuritis treatment trial (ONTT), 304, 306

Optical coherence tomography (OCT), 235

Osteopontin (OPN), 143, 228

Osteoporosis, 160

Paramyxoviruses, 16

Paroxysmal symptoms, 50

PBMC. See Peripheral blood mononuclear cells.

Pemoline (Cylert\textsuperscript{1}), 283

Peripheral blood mononuclear cells (PBMC), 97, 880

Phenotypes, intermediate, 50

Phenotypes, proximal, 50, 54, 56

Picornavirus, 434

Plasma lipoproteins, 50

Platelet activating factor (PAF), 235

PLP. See Proteolipid protein.

Polymerase chain reaction (PCR), 71

Polymorphic polypeptide chains, 43

Polymorphenuclear cell (PMNs), 104

Primary progressive MS (PPMS), 28, 120

Prolactin (PRL), 235

Prolactin receptor (PRLR), 228

Propranolol (Inderal\textsuperscript{1}), 276

Progressive multifocal leukoencephalopathy (PML), 388, 444

Propranolol (Inderal\textsuperscript{1}), 276

Proteolipid protein (PLP), 74, 78, 116

Provigil\textsuperscript{1}, 283
Pseudoexacerbations, 312

Pseudomonas aeruginosa, 72

Recovery, mechanisms of, 201
Region-of-interest (ROI) analysis, 197
Relapsing–remitting MS (RRMS), 25, 301
Remyelination, 125, 454, 487
RRMS. See Relapsing–remitting MS.

Scanning speech, 161
Secondary progressive MS (SPMS), 25, 120
Selective adhesion molecule (SAM) inhibitors, 387
Selective partial inversion recovery prepulse (SPIR), 188
Sensory loss, 292
Sexual symptoms, 165, 275
Single-nucleotide polymorphisms (SNPs), 45
Sisyphean task, 56
Somatosensory evoked potentials, 244–251
Somatosensory symptoms, 159, 160
Spasmolytic agents, 285–287
Spasms, 273, 277
Spasticity, management of, 274, 284, 285
nerve blocks, 287
nociception, 283
spasmolytic agents, 285, 287
stretching, 285
Spinal cord damage, assessment of, 200–201
Spinal cord homogenate (SCH), 360
Split anterior tibial tendon transfer (SPLATT), 287
Stem cell transplantation, autologous, 429
Stem cell transplantation, 426, 427
Stress, effects on disease, 15
Subacute sclerosing panencephalitis (SSPE), 68, 71, 74, 102
Swinging flashlight test, 162
Symmetrel, 283

T1 hypointensity, 491–492
T-cell receptor (TCR), 97, 104, 398, 409
T-cell vaccines, 397, 398, 400
TCR. See T-cell receptor.
Theiler’s murine encephalomyelitis virus (TMEV) disease, 69, 73, 83, 454, 481
Therapeutic plasma exchange (TPE), 302, 308, 310
Th1 to Th2 phenotype, immune deviation from, 862
Thyroid disease, 19
Tissue atrophy, 492, 493
Tissue inhibitor of MMP (TIMP), 230
TMEV. See Theiler’s murine encephalomyelitis virus.
TNF. See Tumor necrosis factor.
Transcription factor, B-cell-specific, 46
Transcutaneous electrical neural stimulation (TENS), 285
Transmission-disequilibrium test (TDT), 54
Tremor, 276, 291, 292
Trigeminal neuralgia, 165
Tumor necrosis factor (TNF-α), 227
Tysabri, 887

Ultra-small superparamagnetic iron oxide (USPIO), 466
Ultraviolet radiation, effects on disease, 14, 15
Universal antigen, 362
University of Pennsylvania Smell Identification Test (UPSIT), 162
Urinary dysfunction, 274

Varicella zoster virus (VZV), 18
Vascular cell adhesion molecule-1 (VCAM-1), 335, 386, 387
Visual dysfunction, 161, 162, 276, 277
Visual evoked potentials (VEPs), 244, 246
Vitamin D receptor gene (VDRG), 14, 15
Voltage gated calcium channels (VGCC), 136

Weakness, 284, 285
Figure 5-1  Chronic multiple sclerosis. (See page 114.)
Figure 5-2  Active multiple sclerosis lesion. (See page 115.)

Figure 5-3  Early active demyelination. (See page 117.)
Figure 5-4  Types of multiple sclerosis plaques. (See page 118.)
Figure 5-5  Inflammation in multiple sclerosis lesions. (See page 119.)

Figure 5-7  Remyelination in chronic multiple sclerosis. (See page 123.)
Figure 5-8
Immunopathological patterns of early multiple sclerosis lesions. (See page 125.)
Figure 5-9  Schematic representation of the four different multiple sclerosis immunopathological subtypes based on the underlying mechanism of myelin/oligodendrocyte destruction. (See page 127.)

Figure 5-10  Axon loss in multiple sclerosis. (See page 132.)
Figure 5-11  Mechanisms of axonal destruction. (See page 133.)

Figure 5-12  Spectrum of inflammatory demyelinating diseases. (See page 138.)
Figure 5-13  Devic disease. (See page 141.)