

Review Article

*Medical Progress***MULTIPLE SCLEROSIS**

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MORE than 100 years has passed since Charcot, Carswell, Cruveilhier, and others described the clinical and pathological characteristics of multiple sclerosis.¹ This enigmatic, relapsing, and often eventually progressive disorder of the white matter of the central nervous system continues to challenge investigators trying to understand the pathogenesis of the disease and prevent its progression.² There are 250,000 to 350,000 patients with multiple sclerosis in the United States.³ Multiple sclerosis typically begins in early adulthood and has a variable prognosis. Fifty percent of patients will need help walking within 15 years after the onset of disease.⁴ Advanced magnetic resonance imaging (MRI) and spectroscopy may allow clinicians to follow the pathological progression of the disease and monitor the response to treatment. Recent progress has occurred in understanding the cause, the genetic components, and the pathologic process of multiple sclerosis. The short-term clinical and MRI manifestations of disease activity have been reduced by new therapies, although the degree of presumed long-term benefit from these treatments will require further study.

CLINICAL COURSE AND DIAGNOSIS

A patient's presenting symptoms and the temporal evolution of the clinical findings may suggest the correct diagnosis. In relapsing–remitting multiple sclerosis — the type present in 80 percent of patients — symptoms and signs typically evolve over a period of several days, stabilize, and then often improve, spontaneously or in response to corticosteroids, within weeks. Relapsing–remitting multiple sclerosis typi-

cally begins in the second or third decade of life and has a female predominance of approximately 2:1. The tendency for corticosteroids to speed recovery from relapses often diminishes with time. Persistent signs of central nervous system dysfunction may develop after a relapse, and the disease may progress between relapses (secondary progressive multiple sclerosis). Twenty percent of affected patients have primary progressive multiple sclerosis, which is characterized by a gradually progressive clinical course and a similar incidence among men and women.

Relapsing–remitting multiple sclerosis typically starts with sensory disturbances, unilateral optic neuritis, diplopia (internuclear ophthalmoplegia), Lhermitte's sign (trunk and limb paresthesias evoked by neck flexion), limb weakness, clumsiness, gait ataxia, and neurogenic bladder and bowel symptoms. Many patients describe fatigue that is worse in the afternoon and is accompanied by physiologic increases in body temperature. The onset of symptoms post partum and symptomatic worsening with increases in body temperature (Uhthoff's symptom) and pseudoexacerbations with fever suggest the diagnosis. Some patients have recurring, brief, stereotypical phenomena (paroxysmal pain or paresthesias, trigeminal neuralgia, episodic clumsiness or dysarthria, and tonic limb posturing) that are highly suggestive of multiple sclerosis.

Prominent cortical signs (aphasia, apraxia, recurrent seizures, visual-field loss, and early dementia) and extrapyramidal phenomena (chorea and rigidity) only rarely dominate the clinical picture. Eventually, cognitive impairment, depression, emotional lability, dysarthria, dysphagia, vertigo, progressive quadriparesis and sensory loss, ataxic tremors, pain, sexual dysfunction, spasticity, and other manifestations of central nervous system dysfunction may become troublesome. Patients who have primary progressive multiple sclerosis often present with a slowly evolving upper-motor-neuron syndrome of the legs ("chronic progressive myelopathy"). Typically, this variant worsens gradually, and quadriparesis, cognitive decline, visual loss, brain-stem syndromes, and cerebellar, bowel, bladder, and sexual dysfunction may develop.

The diagnosis is based on established clinical and, when necessary, laboratory criteria.⁵ Advances in cerebrospinal fluid analysis and MRI, in particular, have simplified the diagnostic process (Fig. 1).⁶ The relapsing forms are considered clinically definite when neurologic dysfunction becomes "disseminated in space and time." Primary progressive multiple sclerosis may be suggested clinically by a progressive course that lasts longer than six months, but laboratory studies to obtain supportive evidence and efforts to exclude

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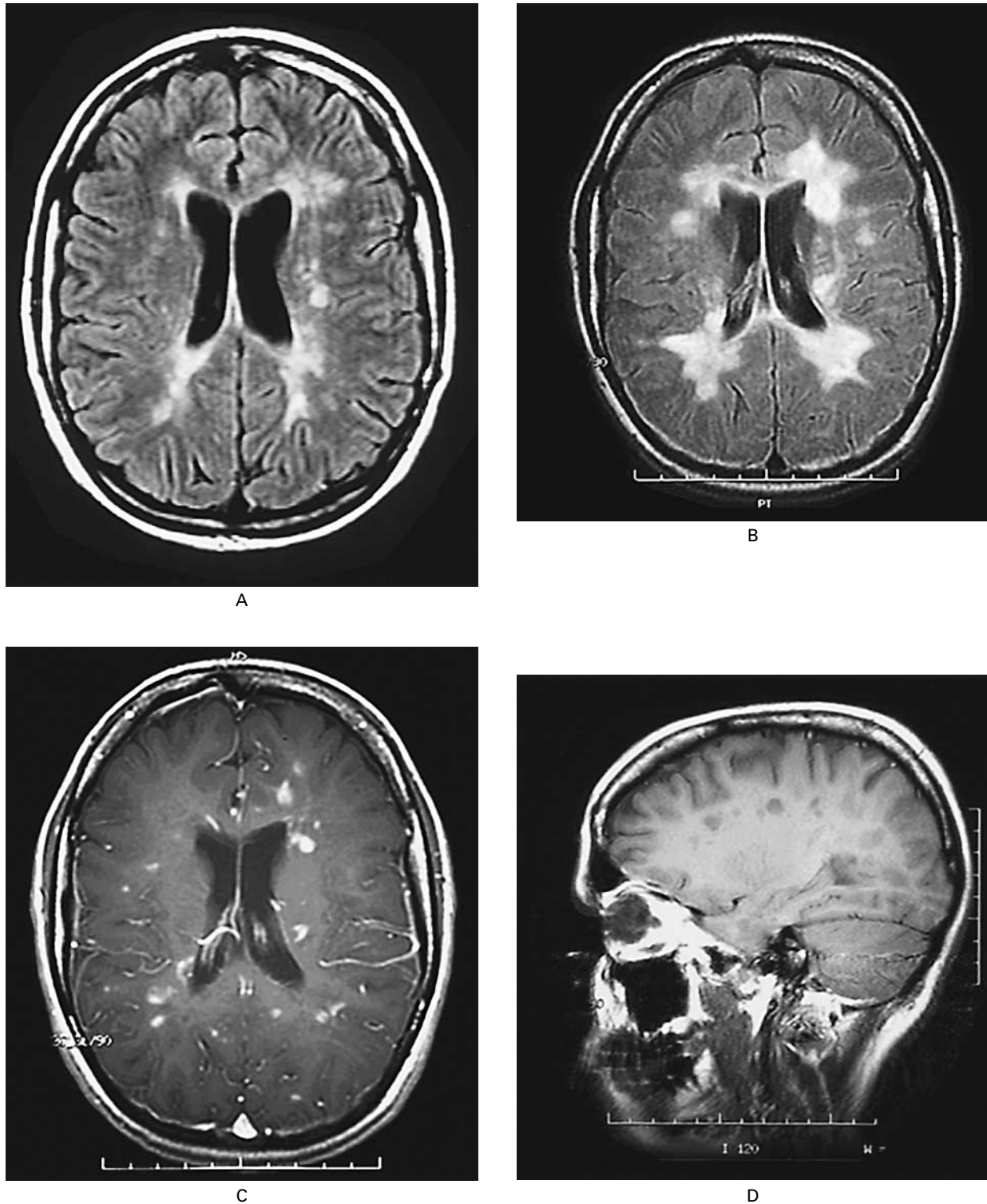


Figure 1. MRI Scans of the Brain of a 25-Year-Old Woman with Relapsing-Remitting Multiple Sclerosis.

An axial FLAIR (fluid-attenuated inversion recovery) image shows multiple ovoid and confluent hyperintense lesions in the periventricular white matter (Panel A). Nine months later, the number and size of the lesions have substantially increased (Panel B). After the administration of gadolinium, many of the lesions demonstrate ring or peripheral enhancement, indicating the breakdown of the blood-brain barrier (Panel C). In Panel D, a parasagittal T₁-weighted MRI scan shows multiple regions in which the signal is diminished (referred to as “black holes”) in the periventricular white matter and corpus callosum. These regions correspond to the chronic lesions of multiple sclerosis.

other, potentially treatable illnesses are advised; for example, structural or metabolic myelopathy can be identified by appropriate laboratory studies, including spinal MRI (Table 1). On MRI, findings of multifocal lesions of various ages, especially those involving the periventricular white matter, brain stem, cerebellum, and spinal cord white matter, support the clinical impression. The presence of gadolinium-enhancing lesions on MRI indicates current sites of presumed inflammatory demyelination (active lesions).

When there is diagnostic uncertainty, repeated MRI after several months may provide evidence that the lesions are "disseminated in time." Cerebrospinal fluid analysis often shows increased intrathecal synthesis of immunoglobulins of restricted specificity (oligoclonal bands may be present, or the synthesis of IgG may be increased), with moderate lymphocytic pleocytosis (almost invariably there are fewer than 50 mononuclear cells). Physiologic evidence of subclinical dysfunction of the optic nerves and spinal cord (changes in visual evoked responses and somatosensory evoked potentials) may provide support for the conclusion that there is "dissemination in space."⁷ Therefore, spinal MRI and evoked-potential testing may provide evidence of a second lesion that can confirm the diagnosis. Abnormalities detected by testing of somatosensory evoked potentials and spinal MRI may clarify the diagnosis in patients with optic neuritis alone or isolated brain-stem abnormalities and in those suspected of having unifocal cerebral multiple sclerosis on the basis of MRI. If positive, abnormalities detected by tests of visual evoked responses may support the diagnosis of multiple sclerosis in patients with isolated brain-stem or spinal cord lesions.

The course of multiple sclerosis in an individual patient is largely unpredictable. Patients who have a so-called clinically isolated syndrome (e.g., optic neuritis, brain-stem dysfunction, or incomplete transverse myelitis) as their first event have a greater risk of both recurrent events (thereby confirming the diagnosis of clinically definite multiple sclerosis) and disability within a decade if changes are seen in clinically asymptomatic regions on MRI of the brain.⁸ The presence of oligoclonal bands in cerebrospinal fluid slightly increases the risk of recurrent disease.⁹

Studies of the natural history of the disease have provided important prognostic information that is useful for counseling patients and planning clinical trials.^{4,10,11} Ten percent of patients do well for more than 20 years and are thus considered to have benign multiple sclerosis. Approximately 70 percent will have secondary progression.⁴ Frequent relapses in the first two years, a progressive course from the onset, male sex, and early, permanent motor or cerebellar findings are independently, but imperfectly, predictive of a more severe clinical course. Women and patients with predominantly sensory symptoms and optic neu-

TABLE 1. DIFFERENTIAL DIAGNOSIS OF MULTIPLE SCLEROSIS.

Metabolic disorders
Disorders of B ₁₂ metabolism* Leukodystrophies
Autoimmune diseases
Sjögren's syndrome, systemic lupus erythematosus, Behçet's disease, sarcoidosis, chronic inflammatory demyelinating polyradiculopathy associated with central nervous system demyelination, antiphospholipid-antibody syndrome
Infections†
HIV-associated myelopathy* and HTLV-1-associated myelopathy,* Lyme disease, meningovascular syphilis, Eales' disease
Vascular disorders
Spinal dural arteriovenous fistula* Cavernous hemangiomas Central nervous system vasculitis, including retinocochlear cerebral vasculitis Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
Genetic syndromes
Hereditary ataxias and hereditary paraplegias* Leber's optic atrophy and other mitochondrial cytopathies
Lesions of the posterior fossa and spinal cord
Arnold-Chiari malformation, nonhereditary ataxias Spondylotic and other myelopathies*
Psychiatric disorders
Conversion reaction, malingering
Neoplastic diseases
Spinal cord tumors,* central nervous system lymphoma Paraneoplastic disorders
Variants of multiple sclerosis‡
Optic neuritis; isolated brain-stem syndromes; transverse myelitis; acute disseminated encephalomyelitis, Marburg disease; neuromyelitis optica

*This disorder or group of disorders is of particular relevance in the differential diagnosis of progressive myelopathy and primary progressive multiple sclerosis.

†HIV denotes human immunodeficiency virus, and HTLV-1 human T-cell lymphotropic virus type 1.

‡In many patients with these variants, clinically definite multiple sclerosis develops or the course is indistinguishable from that of multiple sclerosis.

ritis have a more favorable prognosis. Life expectancy may be shortened slightly; in rare cases, patients with fulminant disease die within months after the onset of multiple sclerosis. Suicide remains a risk, even for young patients with mild symptoms.¹²

EPIDEMIOLOGIC FEATURES

The prevalence of multiple sclerosis varies considerably around the world.¹³ Kurtzke classified regions of the world according to prevalence: a low prevalence was considered less than 5 cases per 100,000 persons, an intermediate prevalence was 5 to 30 per 100,000 persons, and a high prevalence was more than 30 per 100,000 persons.¹⁴ The prevalence is highest in northern Europe, southern Australia, and the middle part of North America. There has been

a trend toward an increasing prevalence and incidence, particularly in southern Europe.^{15,16} Even in areas with uniform methods of ascertainment and high prevalence, such as Olmsted County, Minnesota, the incidence has increased from 2 to 6 per 100,000 during the past century.¹⁷ However, the incidence has actually declined in some,^{18,19} but not all,²⁰ areas of northern Europe. Stable or declining rates have been reported most often in regions with high prevalence and incidence. The extent to which the observed increases in incidence are explained by an enhanced awareness of the disease and improved diagnostic techniques is uncertain. There is a large reservoir of mild cases, the recognition of which may depend heavily on the zeal and resources of the investigator.

The reasons for the variation in the prevalence and incidence of multiple sclerosis worldwide are not understood. Environmental and genetic explanations have been offered, and both factors probably have a role. The occurrence of rapid shifts in the incidence of multiple sclerosis, if not artifactual, is an argument for an environmental influence, as is the equivocal, but suggestive, evidence of the clustering of cases in terms of both geography and time and of epidemics, especially on the Faroe Islands.²¹ The apparent change in the frequency of multiple sclerosis among people^{22,23} and their offspring²⁴ who migrate to and from high-prevalence areas is another factor that has been presented to support the existence of an environmental factor. However, each of these relations has potential confounders that preclude the drawing of a definite conclusion regarding the importance of environmental factors.²⁵ The nature of putative environmental factors remains unclear in numerous case-control studies. Studies that show that the incidence of multiple sclerosis among the adopted children of patients with multiple sclerosis is not higher than expected seem to argue against the possibility that a transmissible factor is primarily responsible for the increased risk of the disease among relatives and instead suggest that genetic factors may be responsible.²⁶

GENETIC FACTORS

Evidence that genetic factors have a substantial effect on susceptibility to multiple sclerosis is unequivocal. The concordance rate of 31 percent among monozygotic twins is approximately six times the rate among dizygotic twins (5 percent).²⁷ The absolute risk of the disease in a first-degree relative of a patient with multiple sclerosis is less than 5 percent; however, the risk in such relatives is 20 to 40 times the risk in the general population.²⁸ Since 1973, it has been recognized that the presence of the HLA-DR2 allele substantially increases the risk of multiple sclerosis.²⁹ This effect has been found in all populations, with the exception of that in Sardinia.³⁰ The magnitude of the relative risk depends on the frequency of the HLA-DR2 allele in the general population. Given the high

frequency of this allele in the population, the risk attributable to the HLA-DR2 allele is considerable. Populations with a high frequency of the allele (e.g., those in Scotland) have the highest risk of multiple sclerosis.

The mode of transmission of genetic susceptibility to multiple sclerosis is complex. Most cases are sporadic, despite the clear excess risk among the relatives of patients. Investigators have used the usual genetic approaches to identify genes associated with an increased risk of multiple sclerosis.

Studies of candidate genes have targeted individual genes with microsatellite markers with use of association and linkage strategies. For some genetic regions, such as the HLA region on chromosome 6, it has been difficult to identify the specific polymorphism that predisposes persons to the disease, given the high degree of linkage disequilibrium at that locus. Candidate-gene studies were followed by four studies in which the entire genome was scanned.³¹⁻³⁴ Regions of interest have been identified, although none have been linked to the disease with certainty. Considering the rather large number of patients evaluated in such studies, one might conclude tentatively that no single gene, except possibly those for HLA antigens,³⁵ exerts a strong effect.

Further refinement of the linkage map is in progress.³⁶ Whether this approach will prove powerful enough to identify genes with a relatively weak effect is difficult to predict. To enhance the detection of genes with a weak effect, investigators have begun to use strategies involving linkage-disequilibrium mapping and transmission-disequilibrium testing. In these approaches, putative causative alleles or marker alleles and haplotypes are assessed to determine whether they are associated with the disease at a population level or whether they are associated with a higher-than-expected rate of transmission of disease from heterozygous parents to their children. This effort will involve a major expenditure of resources to achieve genome-wide coverage. The development of novel analytic techniques for these types of genetic data sets makes such an undertaking feasible.³⁷

The severity and course of multiple sclerosis may also be influenced by genetic factors. Epidemiologic evidence to support this premise comes from studies examining the rate of concordance for measures that describe and quantitate variations in the course of disease, including the age at onset, the proportion of patients in whom the disease progresses, and the extent of disability over time.³⁸ HLA-DR and DQ polymorphisms are not associated with the course and severity of multiple sclerosis, despite their substantial contribution to disease susceptibility.³⁹ Recently, variants of the interleukin-1 β -receptor and interleukin-1-receptor antagonist genes,⁴⁰ immunoglobulin Fc receptor genes,⁴¹ and apolipoprotein E gene⁴² have been associated with the course of the disease, but these findings await confirmation.

PATHOLOGICAL FEATURES AND PATHOGENESIS

Multiple sclerosis is generally believed to be an immune-mediated disorder that occurs in genetically susceptible people (Fig. 2).⁴³ However, the sequence of events that initiates the disease remains largely unknown. Given the considerable clinical, genetic, MRI, and pathological heterogeneity of multiple sclerosis, perhaps more than one pathogenetic mechanism contributes to tissue injury. This possibility has therapeutic implications, because more than one approach to treatment may be required to treat this disease effectively.

The pathological hallmark of chronic multiple sclerosis is the demyelinated plaque, which consists of a well-demarcated hypocellular area characterized by the loss of myelin, relative preservation of axons, and the formation of astrocytic scars (Fig. 3). Lesions have a predilection for the optic nerves, periventricular white matter, brain stem, cerebellum, and spinal cord white matter, and they often surround one or several medium-sized vessels. Although the lesions are usually round or oval, they often have finger-like extensions along the path of small or medium-sized blood vessels (Dawson's fingers). Inflammatory cells are typically perivascular in location, but they may diffusely infiltrate the parenchyma. The composition of the inflammatory infiltrate varies depending on the stage of demyelinating activity. In general, it is composed

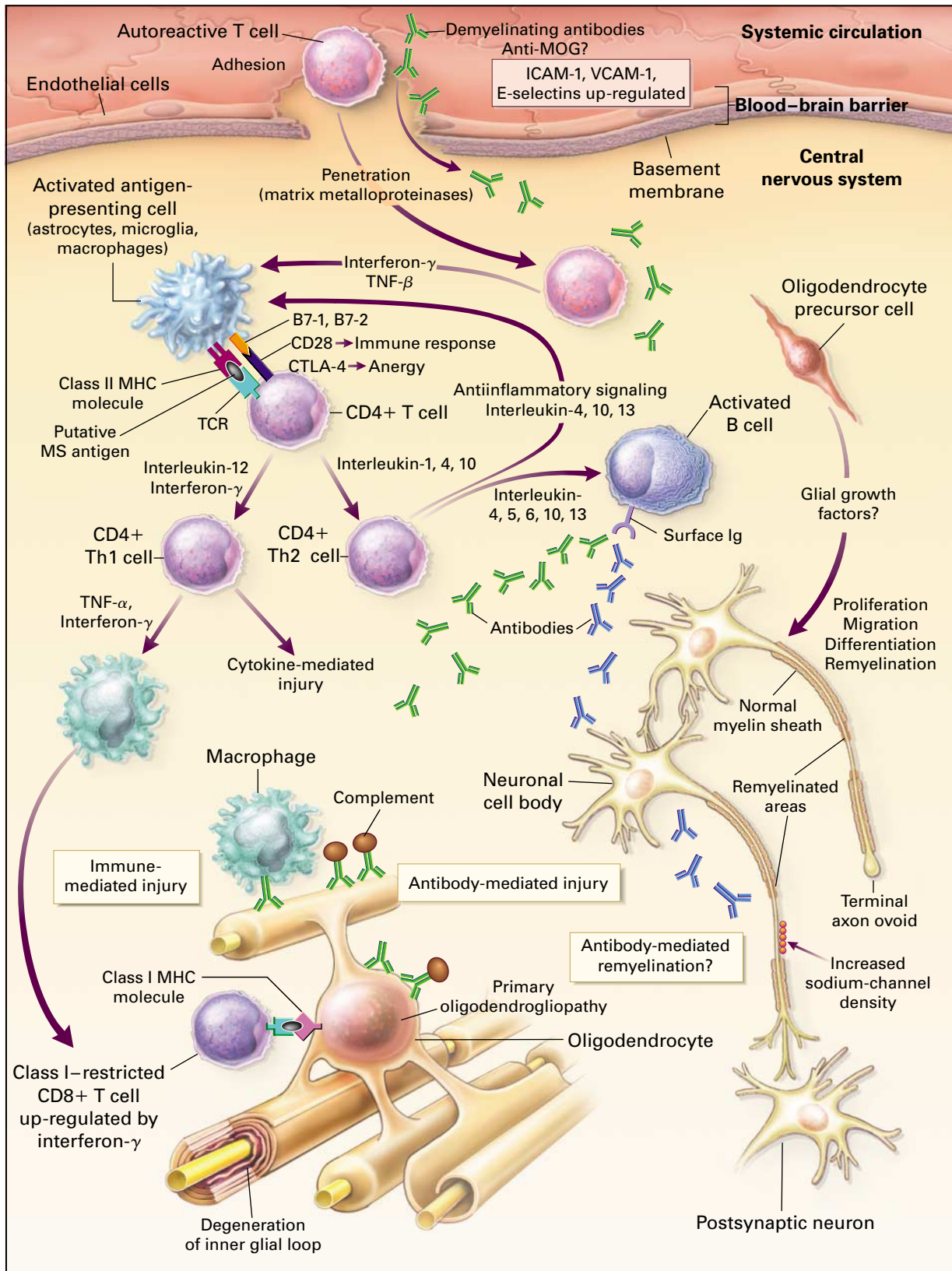
of lymphocytes and macrophages; the latter predominate in active lesions.

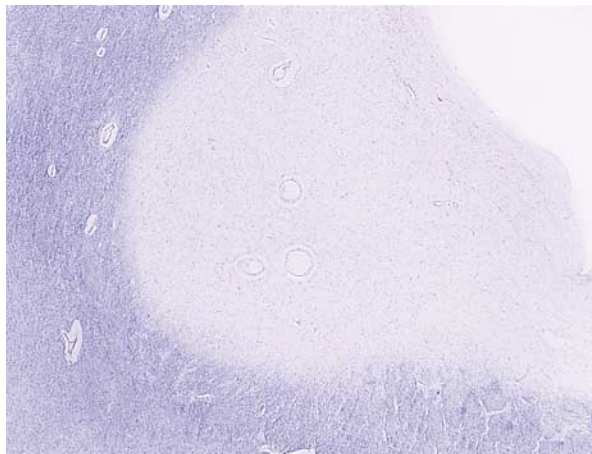
For meaningful conclusions to be drawn regarding the earliest immunologic and molecular events contributing to the formation of lesions, only actively demyelinating plaques should be considered. Identifying myelin-degradation products in macrophages is the most reliable method of identifying active lesions (Fig. 4).⁴⁴ When stringent criteria are used to define lesional activity, the frequency of active plaques in patients with chronic multiple sclerosis is extremely low. Although remyelination is minimal in lesions associated with chronic multiple sclerosis, plaques in acute and early multiple sclerosis may have extensive remyelination (referred to as shadow plaques) (Fig. 5). Furthermore, the lesions of chronic multiple sclerosis reportedly contain substantial numbers of oligodendrocyte precursor cells.⁴⁵ Thus, central nervous system myelin can be repaired, and mechanisms that promote endogenous remyelination may represent a feasible therapeutic strategy.

Early symptoms of multiple sclerosis are widely believed to result from axonal demyelination, which leads to the slowing or blockade of conduction. The regression of symptoms has been attributed to the resolution of inflammatory edema and to partial remyelination. However, inflammatory cytokines may inhibit axonal function, and the recovery of function

Figure 2 (facing page). Possible Mechanisms of Injury and Repair in Multiple Sclerosis.

Genetic and environmental factors (including viral infection, bacterial lipopolysaccharides, superantigens, reactive metabolites, and metabolic stress) may facilitate the movement of autoreactive T cells and demyelinating antibodies from the systemic circulation into the central nervous system through disruption of the blood-brain barrier. In the central nervous system, local factors (including viral infection and metabolic stress) may up-regulate the expression of endothelial adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1), vascular-cell adhesion molecule 1 (VCAM-1), and E-selectin, further facilitating the entry of T cells into the central nervous system. Proteases, including matrix metalloproteinases, may further enhance the migration of autoreactive immune cells by degrading extracellular-matrix macromolecules. Proinflammatory cytokines released by activated T cells, such as interferon- γ and tumor necrosis factor β (TNF- β), may up-regulate the expression of cell-surface molecules on neighboring lymphocytes and antigen-presenting cells. Binding of putative multiple sclerosis (MS) antigens, such as myelin basic protein, myelin-associated glycoprotein, myelin oligodendrocyte glycoprotein (MOG), proteolipid protein, α B-crystallin, phosphodiesterases, and S-100 protein, by the trimolecular complex — the T-cell receptor (TCR) and class II major-histocompatibility-complex (MHC) molecules on antigen-presenting cells — may trigger either an enhanced immune response against the bound antigen or anergy, depending on the type of signaling that results from interactions with surface costimulatory molecules (e.g., CD28 and CTLA-4) and their ligands (e.g., B7-1 and B7-2). Down-regulation of the immune response (anergy) may result in the release of antiinflammatory cytokines (interleukin-1, interleukin-4, and interleukin-10) from CD4+ T cells, leading to the proliferation of antiinflammatory CD4+ type 2 helper T (Th2) cells. Th2 cells may send antiinflammatory signals to the activated antigen-presenting cells and stimulate pathologic or repair-enhancing antibody-producing B cells. Alternatively, if antigen processing results in an enhanced immune response, proinflammatory cytokines (e.g., interleukin-12 and interferon- γ) may trigger a cascade of events, resulting in the proliferation of proinflammatory CD4+ type 1 helper T (Th1) cells and ultimately in immune-mediated injury to myelin and oligodendrocytes. Multiple mechanisms of immune-mediated injury of myelin have been postulated: cytokine-mediated injury of oligodendrocytes and myelin; digestion of surface myelin antigens by macrophages, including binding of antibodies against myelin and oligodendrocytes (i.e., antibody-dependent cytotoxicity); complement-mediated injury; and direct injury of oligodendrocytes by CD4+ and CD8+ T cells. This injury to the myelin membrane results in denuded axons that are no longer able to transmit action potentials efficiently within the central nervous system (loss of saltatory conduction). This slowing or blocking of the action potential results in the production of neurologic symptoms. The exposed axon segments may be susceptible to further injury from soluble mediators of injury (including cytokines, chemokines, complement, and proteases), resulting in irreversible axonal injury (such as axonal transection and terminal axon ovoids). There are several possible mechanisms of repair of the myelin membrane, including resolution of the inflammatory response followed by spontaneous remyelination, spread of sodium channels from the nodes of Ranvier to cover denuded axon segments and restore conduction, antibody-mediated remyelination, and remyelination resulting from the proliferation, migration, and differentiation of resident oligodendrocyte precursor cells. Adapted from a drawing by the Mayo Foundation.





A



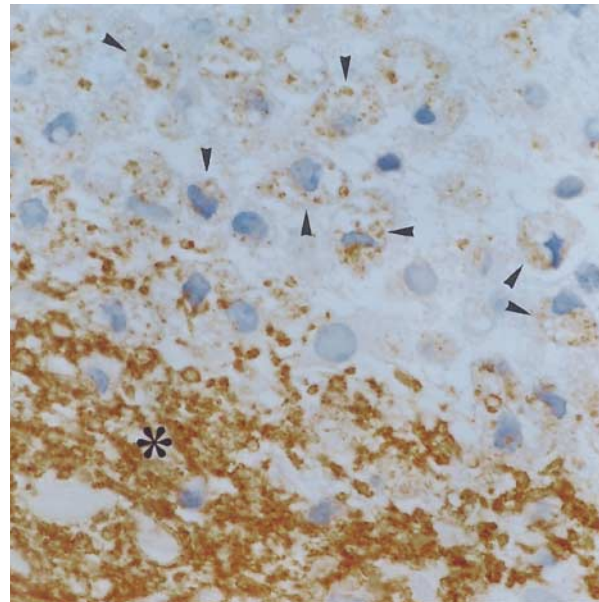
B

Figure 3. Photomicrographs of a Chronic Multiple Sclerosis Plaque.

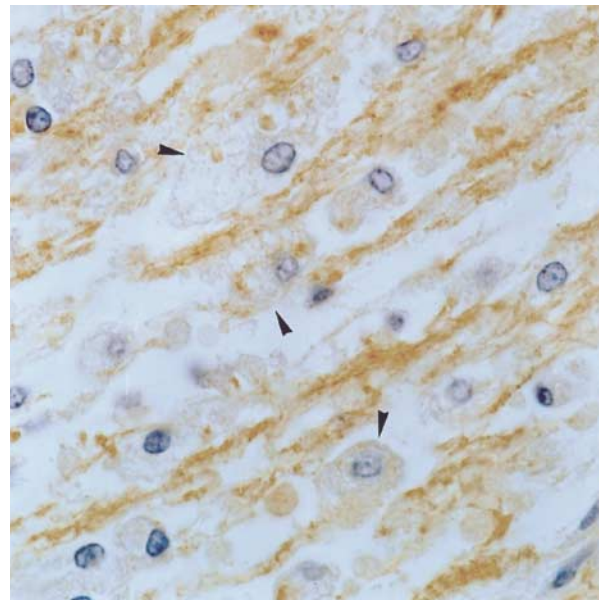
In Panel A, a well-demarcated hypocellular region of myelin loss is evident in the periventricular white matter (luxol fast blue and periodic acid-Schiff myelin stain, $\times 15$). In Panel B, neurofilament staining for axons in the same lesion demonstrates a reduction in axonal density ($\times 15$).

may result from the redistribution of sodium channels across segments of demyelinated axons.^{46,47} Irreversible axonal injury, gliotic scarring, and exhaustion of the oligodendrocyte progenitor pool may result from repeated episodes of disease activity and lead to progressive loss of neurologic function. Axonal injury may occur not only in the late phases of multiple sclerosis but also after early episodes of inflammatory demyelination.⁴⁸⁻⁵⁰ The pathogenesis of this early axonal injury is still unclear.

Experimental *in vitro* and *in vivo* models of inflammatory demyelination suggest that diverse disease processes, including autoimmunity and viral infection,



A



B

Figure 4. Photomicrographs of an Actively Demyelinating Multiple Sclerosis Lesion (Immunocytochemical Staining of Myelin Oligodendrocyte Glycoprotein [Brown] with Hematoxylin Counterstaining of Nuclei [Blue]).

In Panel A, at the active edge of a multiple sclerosis lesion (indicated by the asterisk), the products of myelin degradation are present in numerous macrophages (arrowheads) ($\times 100$). In Panel B ($\times 100$), macrophages containing myelin debris (arrowheads) are interdigitated with degenerating myelin sheaths.

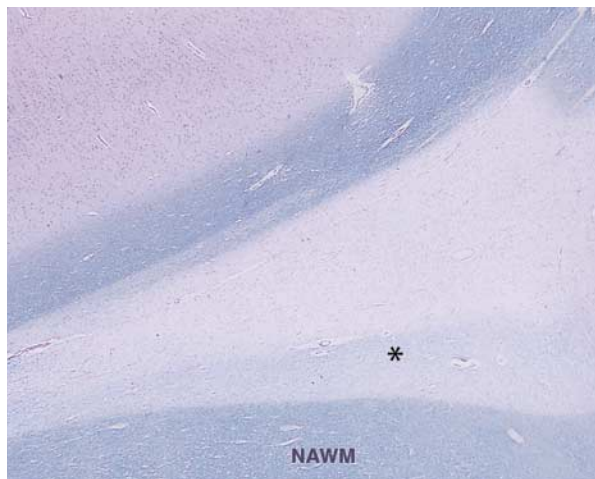


Figure 5. Remyelination in a Lesion Associated with Chronic Multiple Sclerosis.

The area stained pale blue (indicated by the asterisk) represents a region of partial remyelination (a shadow plaque) along the periventricular edge of a lesion in a patient with chronic multiple sclerosis (luxol fast blue and periodic acid–Schiff myelin stain, $\times 15$). NAWM denotes normal-appearing white matter.

may induce multiple sclerosis–like inflammatory demyelinated plaques. Activated CD4+ T cells specific for one or more self antigens are believed to adhere to the luminal surface of endothelial cells in central nervous system venules and migrate into the central nervous system at the time of disruption of the blood–brain barrier. This process is followed by an amplification of the immune response after the recognition of target antigens on antigen-presenting cells. The existence of T cells that are reactive to several putative self myelin and non-myelin “multiple sclerosis antigens,” including myelin basic protein, myelin-associated glycoprotein, myelin oligodendrocyte glycoprotein, proteolipid protein, $\alpha\beta$ -crystallin, phosphodiesterases, and S-100 protein, has been proposed.^{51–53} Additional amplification factors including autoantibodies or cytokines may also be necessary to produce the demyelinated plaque^{2,54} (Fig. 2).

Antibodies against antigens located on the surface of the myelin sheath or oligodendrocyte can cause demyelination directly, possibly through the activation of complement, leading to complement-mediated cytotoxicity.⁵⁵ These antibodies may gain access to the central nervous system through the disruption of the blood–brain barrier as a consequence of a T-cell-initiated inflammatory response. The existence of antibody-mediated demyelination is supported in part by the observation that demyelination was augmented by the administration of antibody specific for myelin oligodendrocyte glycoprotein to rats with experimentally induced allergic encephalomyelitis⁵⁶ (the glyco-

protein is present on the outer lamellae of the myelin sheath). Antibodies against both myelin oligodendrocyte glycoprotein and myelin basic protein can be found in the brains of patients with multiple sclerosis.⁵⁷ Deposits of immunoglobulin and activated complement may be present in multiple sclerosis lesions in which myelin is being degraded.⁵⁸ Taken together, these observations suggest that an antibody-mediated process may have an important role in the pathogenesis of multiple sclerosis.

Other factors may also help degrade myelin and damage oligodendrocytes. Activated macrophages and microglial cells may mediate such activity by producing proinflammatory cytokines (such as tumor necrosis factor α and interferon- γ), generating reactive oxygen or nitrogen species, producing excitatory amino acids, activating complement components, or releasing proteolytic and lipolytic enzymes. Other factors potentially toxic to oligodendroglial cells include soluble T-cell products (such as perforin), the interaction of Fas antigen with Fas ligand, cytotoxicity mediated by the interaction of CD8+ T cells with class I major-histocompatibility-complex (MHC) antigens on antigen-presenting cells, and persistent viral infection.⁵⁴ Human herpesvirus type 6 can cause a condition that mimics multiple sclerosis⁵⁹ and appears in oligodendrocytes within multiple sclerosis tissue in some patients, but not in control tissue.⁶⁰ A direct causal link, however, remains to be confirmed. In one study, *Chlamydia pneumoniae* was isolated from 64 percent of patients with multiple sclerosis, as compared with 11 percent of control patients with other neurologic diseases, and it was detected in cerebrospinal fluid by a polymerase-chain-reaction assay in 97 percent of patients with multiple sclerosis, as compared with 18 percent of control patients.⁶¹ These results have yet to be confirmed in other laboratories.⁶²

Various pathogenic mechanisms may be involved in multiple sclerosis. There is an important degree of variability among patients in the structural and immunologic features of the lesions of multiple sclerosis.⁶³ The extent of survival of oligodendrocytes varies from patient to patient but is uniform within a given patient, suggesting that the focus of injury (myelin, mature oligodendrocyte, or progenitor cell) varies among patients.⁴⁹ Although most lesions are characterized by an inflammatory reaction, composed mainly of T lymphocytes and macrophages, diverse patterns of myelin destruction have been described.⁶⁴

In some lesions, the presence of immunoglobulins and activated terminal complement components suggests that demyelinating antibodies have a pathogenic role. In others, a primary oligodendrocyte dystrophy manifested by the selective loss of myelin-associated glycoprotein and apoptosis of oligodendrocytes has been seen. Finally, in other cases, a small rim of necrotic oligodendrocytes has been found in the nor-

mal-appearing white matter adjacent to the active plaque edge. The patterns of demyelination were heterogeneous among patients, but homogeneous within active plaques from the same patient. Multiple sclerosis may therefore be a series of syndromes with different causes and pathogenic mechanisms (e.g., cellular-mediated immune injury, complement- and antibody-mediated injury, or primary oligodendroglial dystrophy). If confirmed, this possibility could lead to the identification of markers of the underlying pathologic processes that could be used to individualize treatment.

MRI and spectroscopy may be helpful in characterizing the underlying pathologic processes in multiple sclerosis.⁶ There is consensus that T₂-weighted MRI reflects a broad spectrum of pathological changes, including inflammation, edema, demyelination, gliosis, and axonal loss. Changes in the number and volume of lesions on T₂-weighted MRI (referred to as the T₂-weighted lesion load) are sensitive but nonspecific indicators of disease activity and the response to treatment. New lesions and areas of gadolinium enhancement on T₁-weighted MRI suggest recent inflammatory demyelination with disruption of the blood-brain barrier (Fig. 1). Monitoring by means of serial MRI studies with gadolinium enhancement helps to identify agents that may be active against this early inflammatory stage of multiple sclerosis (e.g., corticosteroids, interferons, glatiramer acetate, and certain immunosuppressive agents).⁶⁵⁻⁶⁹

There is MRI and pathological evidence that the normal-appearing white matter is not normal in patients with multiple sclerosis.^{70,71} Serial MRI studies of normal-appearing white matter may be useful to determine where abnormalities are likely to develop.⁷² Findings of “black holes” on T₁-weighted images, changes in magnetization-transfer ratios (a measure of free and bound water, which is an indication of the degree of structural disruption) (Fig. 1), and serial decreases in the volume of the brain and spinal cord (indicating atrophy) on imaging studies most likely correlate with both the loss of axons and the occurrence of extensive demyelination; these may ultimately be useful markers of the late, secondary degenerative phase of the illness. These measures, along with MRI spectroscopic markers of the number and function of neurons (e.g., the levels of *N*-acetyl aspartate), may eventually prove to be valid, objective surrogate measures of axonal abnormalities.

TREATMENT

Principles of Therapy

Patients with multiple sclerosis face enormous prognostic uncertainty, and they must become well informed about their illness. This is perhaps best accomplished with a multidisciplinary approach involving a neurologist, an allied health worker (e.g., nurse or a social worker) with expertise in multiple sclerosis,

and information from national and local multiple sclerosis organizations. Treating physicians must continually assess the need for psychological support for patients and their families, since depression is common and the rate of suicide is relatively high in this population of patients.¹²

Physicians and patients need to distinguish clinical relapses from the transient worsening of symptoms that may accompany an increase in body temperature or fatigue. Patients should be reassured that findings of recent disease activity do not invariably indicate an unfavorable long-term prognosis and that pregnancy does not worsen the long-term outcome.⁷³ Patients should limit their exposure to viral illnesses because infections may trigger relapses.⁷⁴ Vaccinations may be safely administered to patients who may be at risk for influenza.⁷⁵ Because of reports that the hepatitis B virus vaccine may trigger multiple sclerosis, this vaccine should be administered only to persons at substantial risk of exposure to the virus — until the relative risks associated with vaccination are clarified by definitive, prospective studies that include MRI.

Relapses

Corticosteroids are often used to treat clinically significant relapses in an attempt to hasten recovery; for example, intravenous methylprednisolone may be given for five days, followed by an optional brief course of prednisone. There is no consensus about the optimal form, dose, route, or duration of corticosteroid therapy (Table 2). Other experimental strategies⁷⁸ have not proved to be better than corticosteroids. A post hoc analysis of the Optic Neuritis Treatment Trial suggested that prednisone might increase the risk of recurrent episodes of disease activity⁷⁹ and that early intervention with intravenous methylprednisolone and prednisone delayed the recurrence of neurologic events for two years.⁸⁰ These findings changed clinical practice: oral prednisone is now rarely used to treat acute optic neuritis.

A recent, double-blind, crossover trial demonstrated that a regimen of seven alternate-day plasma exchanges was followed by substantial clinical improvement in approximately 40 percent of patients who had catastrophic episodes of inflammatory demyelination that were unresponsive to corticosteroids.⁸¹ These results require confirmation.

The optimal treatment of patients after a first clinical episode of possible multiple sclerosis remains uncertain. As discussed earlier, the risk of recurrence and the extent of disability can to some extent be predicted by the findings on MRI of the brain at the time of the first clinical episode.⁸ Two recently completed phase 3 trials^{82,83} suggest that treatment with interferon beta-1a may delay the development of a second, diagnosis-defining bout (clinically definite multiple sclerosis). In this issue of the *Journal*, Jacobs et al.⁸³ report that early treatment with interferon beta-1a

TABLE 2. CURRENT TREATMENTS FOR MULTIPLE SCLEROSIS.

TYPE OF MULTIPLE SCLEROSIS OR RELAPSE	AGENT	DOSE	KNOWN OR POSSIBLE BENEFITS OF TREATMENT	UNKNOWN EFFECTS OR ASPECTS OF TREATMENT
Relapsing–remitting	Interferon beta-1b (Betaseron)	8 million IU subcutaneously every other day	Reduces rate of clinical relapse Reduces the development of new lesions on MRI Delays the increase in the volume of lesions on MRI	Ability to delay progression of disability Duration and clinical significance of benefit Mechanism of action Most effective dose and route of administration Frequency and clinical significance of the formation of neutralizing antibodies
	Interferon beta-1a (Avonex)	30 µg intramuscularly once weekly	Reduces rate of clinical relapse May delay progression of disability Reduces the development of new lesions on MRI Delays the increase in the volume of lesions on MRI	Whether the effect on disability is clinically meaningful and sustained Duration and clinical significance of benefit Mechanisms of action Most effective dose and route of administration Frequency and clinical significance of the formation of neutralizing antibodies
	High-dose interferon beta-1a (Rebif)*	22 or 44 µg subcutaneously every other day	Possible dose-related benefit in patients with more severe disabilities	
	Glatiramer acetate (Copaxone)	20 µg subcutaneously daily	Reduces rate of clinical relapse Moderately reduces the development of new lesions on MRI	Effect on the progression of disability Duration and clinical significance of benefit Mechanism of action Most effective dose and route of administration
	Immune globulin	0.15–0.2 g/kg of body weight intravenously monthly for 2 yr	Reduces rate of clinical relapse May delay progression of disability	Whether progression of disability is actually delayed, as measured by a second evaluation in 3 mo Effect on the number and volume of lesions, as assessed by MRI Duration and clinical significance of benefit Mechanism of action Most effective dose and route of administration
Secondary progressive	Interferon beta-1b (Betaferon)	8 million IU subcutaneously every other day	Reduces rate of clinical relapse May reduce progression of disability regardless of relapse status (recent or current)† Delays the increase in the volume of lesions on MRI	Whether progression of disability is actually delayed, and if so, for how long and to what effect Mechanism of action Most effective dose and route of administration Frequency and clinical significance of the formation of neutralizing antibodies
	Mitoxantrone hydrochloride	5 or 12 mg/m ² of body-surface area intravenously every 3 mo for 2 yr	Reduces rate of clinical relapse Delays progression of disability Reduces activity evident on MRI	Duration of benefit Most effective dose Dose-dependent risk of cardiac toxicity
Primary progressive	None			
Acute relapses	Corticosteroids	Various doses (see text)	Hastens clinical recovery Transiently restores blood–brain barrier on MRI	Duration and clinical significance of benefit Effect on progression of disability Mechanism of action Most effective agent, dose, and route of administration Why responsiveness to corticosteroids declines over time
	Plasma exchange	Seven exchanges of one plasma volume on alternate days	Enhances recovery of relapse-related neurologic deficits in patients with no response to high-dose corticosteroids	Effect on recurrent disease Duration of effect Mechanism of action

*This formulation is only available in Canada and Europe.

†This benefit has been observed in one of two studies.^{76,77}

delayed the development of clinical and MRI evidence of recurrent disease in patients with a first demyelinating central nervous system event. This is an expected finding, given the published evidence that interferon beta reduces clinical relapses and changes on MRI scans. This report may influence patients' and physicians' decisions regarding the timing of interferon therapy, although the inconvenience, treat-

ment-related side effects, cost, and lack of evidence of an important long-term benefit of interferon beta will deter others from starting treatment early in the disease course. The relations among inflammatory-mediated demyelination, axonal injury, and clinical disability remain to be clarified. There is a pressing need to determine whether the currently approved, partially effective immunomodulatory therapies re-

duce the degree or delay the development of disability in patients with clinically isolated demyelinating syndromes and definite multiple sclerosis.

Relapsing Multiple Sclerosis

The first of several convincing trials demonstrated that interferon beta-1b (Betaseron, Berlex Laboratories) reduced the frequency of relapse by approximately 30 percent.^{65,66,84} There was also a trend toward a delay in the progression of disability, but this finding did not reach statistical significance. Interferon beta-1a (Avonex, Biogen) and glatiramer acetate (Copaxone, Teva Pharmaceutical Industries)^{85,86} were subsequently found to reduce the frequency of relapse. Interferon beta-1a may delay the progression of disability in patients with minor disability who have a relapsing form of multiple sclerosis.^{67,87,88}

Each of these agents has a number of immunemediating activities; the specific mechanisms of action of these agents in multiple sclerosis are incompletely understood. The interferons reduce the proliferation of T cells and the production of tumor necrosis factor α , decrease antigen presentation, alter cytokine production to favor ones governed by type 2 helper T (Th2) cells, increase the secretion of interleukin-10, and reduce the passage of immune cells across the blood-brain barrier by means of their effects on adhesion molecules, chemokines, and proteases. Glatiramer acetate, formerly known as copolymer-1, is a mixture of synthetic polypeptides containing the L-amino acids glutamic acid, alanine, lysine, and tyrosine. Glatiramer acetate may promote the proliferation of Th2 cytokines; compete with myelin basic protein for presentation on MHC class II molecules, thereby inhibiting antigen-specific T-cell activation (Fig. 2); alter the function of macrophages; and induce antigen-specific suppressor T cells.

All these drugs reduce the development of new, gadolinium-enhancing lesions on MRI with variable effectiveness. All three agents are approved by the Food and Drug Administration (FDA) and are used widely. A higher-dose formulation of interferon beta-1a (Rebif, Ares Serono International) has yet to be approved for use in the United States but is licensed for use in Canada and Europe.⁶⁸ These agents must be administered parenterally, are expensive (each costs approximately \$10,000 per year in the United States), and have variable adverse effects. Their long-term effectiveness has not been established, and studies are now addressing the cost effectiveness of these agents.⁸⁹ Interferon beta-1a and interferon beta-1b may induce the formation of neutralizing antibodies, especially during the first 18 months of treatment. The relevance of neutralizing antibodies, particularly with regard to the level that is clinically significant, is uncertain. There is concern that high titers of neutralizing antibodies may decrease or abrogate the biological activity of interferon beta. It may be advisable to

test patients for neutralizing antibodies if they have no response to interferon beta, although practice guidelines with respect to the interpretation of these tests are not yet available.

Opinions vary on when to initiate treatment with interferon beta and glatiramer acetate. The practice directive of the National Multiple Sclerosis Society states that these agents should be considered in patients with relapsing-remitting multiple sclerosis who have had recent relapses.⁹⁰ Neurologists who initiate treatment when the diagnosis of relapsing-remitting multiple sclerosis is established, or shortly thereafter, believe that these drugs are maximally effective against the early inflammatory phase of the disease. They reason that treatment may limit irreversible axonal injury and delay late deterioration; this hypothesis is based in part on evidence from biopsy studies showing that axonal injury can occur in acute or severe multiple sclerosis.⁴⁸ Other neurologists delay treatment until there is a history of recurrent relapses over a more prolonged period, for a number of reasons. Patients may have a benign early course.⁹¹

Data on the long-term efficacy and safety of these agents are not available. Although axonal injury may occur early, the frequency of early axonal injury is unknown. The formation of neutralizing antibodies may render interferon beta inactive, leaving the patient without this treatment option later in the clinical course. There is no evidence that these agents reduce such injury. The enthusiasm for these treatments, whether started immediately after the diagnosis is made or sometime later, must be tempered by the disappointing reality that most patients continue to have relapses during treatment and ultimately become increasingly disabled.

Patients frequently have firm opinions about the timing and choice of treatment. Given that there are no long-term studies (e.g., ones lasting longer than five years) confirming that any of the agents delay the progression of disability and that there have been no phase 3 comparative studies clarifying which agent is most effective, the treating physician must consider the patient's individual risk of clinically significant early disability and the patient's desire to start or delay treatment. Many North American neurologists initiate treatment after repeated relapses, particularly if the patient's clinical recovery is incomplete. The choice of the specific agent remains highly dependent on the specialist's opinion of its relative potency and the patient's anticipated tolerance of treatment-related side effects. Glatiramer acetate is generally well tolerated and may be most effective for mildly disabled patients with a recent diagnosis of multiple sclerosis who wish to start treatment early in the course of the illness.

Some multiple sclerosis specialists believe that the published evidence favors interferon beta, although the side effects are generally more troublesome than

those of glatiramer acetate. The evidence of a dose–response for interferon beta^{66,68,92,93} may influence the treating physician in the United States to choose interferon beta-1b, which delivers a higher cumulative weekly dose of interferon, rather than interferon beta-1a. (A high-dose formulation of interferon beta-1a [Rebif] is available in Canada and Europe.) Higher doses, however, may be accompanied by more frequent side effects and an increased risk of the formation of neutralizing antibodies. If these factors are considered paramount, the treating physician may choose a lower dose of weekly interferon beta-1a.

One placebo-controlled trial reported that intravenous immune globulin reduced the frequency of relapse.⁹⁴ These results have yet to be confirmed, and immune globulin is not widely used for this indication in North America.

Secondary Progressive Multiple Sclerosis

The indications for the treatment of secondary progressive multiple sclerosis are unclear. Many trials have reported a marginal benefit with various immunosuppressive therapies. A recent phase 3 European trial reported that interferon beta-1b reduced clinical and MRI evidence of disease activity.^{69,76} Treatment delayed the progression of disability regardless of whether relapses occurred before or after randomization, although the magnitude of the effect was moderate. It is not known whether this benefit in patients who were not having ongoing relapses results from an ability of interferon to interfere with the degenerative changes that presumably contribute to the clinical worsening that occurs in most patients after the first decade of the illness. Alternatively, this apparent benefit may reflect an ability of interferon to reduce inflammatory activity, whether manifested clinically or not (e.g., subclinical relapses).

Interferon beta-1b has been approved for use in secondary progressive multiple sclerosis in Europe and Canada. The results of two recently completed phase 3 trials^{77,95,96} indicate that interferon beta-1b and interferon beta-1a may reduce the frequency of relapses and the evidence of disease activity on MRI only in patients who have continual clinical relapses. However, neither of these studies found that treatment slowed the progression of disability. Consequently, the status of interferon beta with respect to the treatment of secondary progressive multiple sclerosis, with or without recent relapses, remains controversial. In a phase 3 European trial, mitoxantrone hydrochloride, an anthracenedione derivative and a cytotoxic agent with associated antiinflammatory activities, reduced both clinical and MRI evidence of disease activity in patients with secondary progressive multiple sclerosis.⁹⁷

Primary Progressive Multiple Sclerosis and the Management of Symptoms

There are no proven therapies for primary progressive multiple sclerosis, although phase 3 trials of

interferons and glatiramer acetate are under way. None of the treatments reverse the neurologic disabilities.

Treatment of Complications

There are moderately effective treatments for several of the complications of multiple sclerosis. Fatigue may respond to amantadine and to energy-conservation strategies. Depression and sleep disorders may contribute to fatigue and must be recognized and treated appropriately. Paroxysmal events typically respond well to carbamazepine and phenytoin (alone or in combination), acetazolamide, gabapentin, and pergolide.

Spasticity, pain, problems with gait, decubitus ulcers, speech and swallowing disorders, and cognitive and mood disorders are best treated by a multidisciplinary approach that may involve specialists in physical medicine and rehabilitation. Stretching, a program of aerobic exercise, and centrally acting muscle relaxants may help patients with mild, symptomatic spasticity. Patients with clinically significant weakness of the legs may require a moderate degree of extensor tone in order to walk and therefore may not be able to tolerate antispasticity medications. The implantation of a pump for the intrathecal administration of baclofen may assist in the management of intractable, painful spasticity in patients who cannot walk and who have lost bowel and bladder function. Neurogenic bladder and bowel disturbances are amenable to treatment after appropriate investigations have clarified the underlying physiologic mechanisms. Sexual dysfunction and chronic, central pain are common and may respond to appropriate symptom-based treatment strategies. Disabling, high-amplitude, cerebellar-outflow tremors rarely respond well to medication but may decrease after continued contralateral thalamic stimulation or ablative thalamotomy.

CHALLENGES IN CONDUCTING CLINICAL TRIALS AND FUTURE DIRECTIONS

Multiple sclerosis remains a challenging disease to study because the cause is unknown, the pathophysiologic mechanisms are diverse, and the chronic, unpredictable course of the disease makes it difficult to determine whether the favorable effects of short-term treatment will be sustained. Most published trials are small (usually including fewer than 150 patients per study group) and brief (less than three years of follow-up⁹⁸). Clinical measures (the degree of disability, the relapse rate, and the time to clinical progression) remain the primary outcomes assessed in phase 3 trials. These measures are relatively insensitive to change and only weakly predictive of the long-term clinical outcome. No laboratory studies, including MRI, meet the requirements of the FDA for a surrogate marker of prognosis.

The important limitations of clinical trials involving patients with chronic illnesses, such as imperfect blinding, a high rate of withdrawal, and an incom-

pletely matched or inappropriate control group, are particularly prominent in studies of multiple sclerosis. In the past several years, trials have used increasingly sophisticated methods to identify promising agents as well as those that are toxic or ineffective.⁹⁹⁻¹⁰¹ Careful attention must be paid to the demographic characteristics of the control group before enrollment and to their clinical behavior after enrollment to avoid false positive results. For example, if the results in the control group are worse than those expected on the basis of the predicted natural history of the disease, the putative benefit of treatment in the other group may be exaggerated.

There is interest in designing trials to assess ways of delaying irreversible axonal injury and promoting remyelination. One strategy would be to evaluate whether combinations of drugs with different mechanisms of action are more effective than single-agent therapy. Other immunomodulating approaches include anticytokine and "immune-deviation" strategies, which are designed to favor the proliferation of anti-inflammatory Th2 cells and Th2 cytokines (Fig. 2). Inhibitors of matrix metalloproteinases and other proteases, inhibitors of cathepsin B, inhibitors and scavengers of oxygen radicals, and efforts to reverse or reduce the activation of the trimolecular complex (including peptide immunotherapy and T-cell vaccination) may be worth additional study. Investigators who favor an infectious cause of multiple sclerosis, such as human herpesvirus type 6⁶⁰ or *C. pneumoniae*,⁶¹ may initiate trials of antiviral and antibacterial agents. Other approaches focusing on reparative and remyelination strategies include efforts to block antibody-mediated demyelination. It may be possible to enhance remyelination by transplanting oligodendroglial precursor cells into discrete, clinically important lesions (e.g., those affecting the optic nerves, the middle cerebellar peduncle, or the spinal cord)^{102,103} while administering growth factors and neuroprotective agents. Gene-therapy strategies may also ultimately be worthy of study.

The widespread use of the partially effective immunomodulatory agents has left few patients who have not received such agents and who would therefore make good candidates for enrollment in trials. It may no longer be ethical to evaluate new treatments for relapsing–remitting multiple sclerosis in a placebo-controlled study except in unusual circumstances, such as those involving patients who have declined standard therapies or who have had no response to them and those involving brief trials in patients with recently diagnosed multiple sclerosis. The costs of definitive trials have also escalated markedly. Phase 3 studies should include at least three years of follow-up to identify biologically meaningful effects of treatment.^{98,104}

During the past decade, there has been moderate progress in reducing the inflammatory component

of multiple sclerosis. Unfortunately, most patients continue to have relapses and progression of their symptoms. This finding has forced a reexamination of the hypothesis that the elimination of acute relapses and, by inference, inflammation would be curative. An alternative hypothesis is that clinical progression is independent of inflammation but depends on factors intrinsic to the pathologic substrate influencing demyelination and, in particular, injury to axons. If this hypothesis is confirmed, newer approaches directed toward interfering with demyelination and axonal injury will be necessary to prevent progression and restore function. Many degenerative neurologic diseases share mechanisms of injury (e.g., apoptosis, oxidative stress, loss of trophic support, and proteolysis). As the margins between the neurodegenerative diseases begin to blur, unifying concepts of nervous system injury will emerge, providing opportunities for the design of rational treatments.

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REFERENCES

1. Compston A, Ebers G, Lassmann H, McDonald I, Matthews B, Wekerle H. *McAlpine's multiple sclerosis*. 3rd ed. London: Churchill Livingstone, 1998.
2. Noseworthy J. Progress in determining the causes and treatment of multiple sclerosis. *Nature* 1999;399:Suppl:A40-A47.
3. Anderson DW, Ellenberg JH, Leventhal CM, Reingold SC, Rodriguez M, Silberberg DH. Revised estimate of the prevalence of multiple sclerosis in the United States. *Ann Neurol* 1992;31:333-6.
4. Weinshenker BG, Bass B, Rice GP, et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. *Brain* 1989;112:133-46.
5. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* 1983;13:227-31.
6. Miller DH, Grossman RI, Reingold SC, McFarland HF. The role of magnetic resonance techniques in understanding and managing multiple sclerosis. *Brain* 1998;121:3-24.
7. Gronseth GS, Ashman EJ. Practice parameter: the usefulness of evoked potentials in identifying clinically silent lesions in patients with suspected multiple sclerosis (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2000;54:1720-5.
8. O'Riordan JI, Thompson AJ, Kingsley DP, et al. The prognostic value of brain MRI in clinically isolated syndromes of the CNS: a 10-year follow-up. *Brain* 1998;121:495-503.
9. Cole SR, Beck RW, Moke PS, Kaufman DI, Tourtellotte WW. The predictive value of CSF oligoclonal banding for MS 5 years after optic neuritis. *Neurology* 1998;51:885-7.
10. Weinshenker BG, Rice GPA, Noseworthy JH, Carriere W, Baskerville J, Ebers GC. The natural history of multiple sclerosis: a geographically based study. 4. Applications to planning and interpretation of clinical therapeutic trials. *Brain* 1991;114:1057-67.
11. Cottrell DA, Kremenchutzky M, Rice GPA, Hader W, Baskerville J, Ebers GC. The natural history of multiple sclerosis: a geographically based study. 6. Applications to planning and interpretation of clinical therapeutic trials in primary progressive multiple sclerosis. *Brain* 1999;122:641-7.
12. Sadovnick AD, Eisen K, Ebers GC, Paty DW. Cause of death in patients attending multiple sclerosis clinics. *Neurology* 1991;41:1193-6.
13. Weinshenker BG, Rodriguez M. *Epidemiology of multiple sclerosis*. In: Gorelick PB, Alter M, eds. *Handbook of neuroepidemiology*. Vol. 29 of *Neurological disease and therapy*. New York: Marcel Dekker, 1994:533-67.

14. Kurtzke JF. Multiple sclerosis: changing times. *Neuroepidemiology* 1991;10:1-8.
15. Rosati G, Aiello I, Pirastru MI, et al. Epidemiology of multiple sclerosis in northwestern Sardinia: further evidence for higher frequency in Sardinians compared to other Italians. *Neuroepidemiology* 1996;15:10-9.
16. Bufill E, Blesa R, Galan I, Dean G. Prevalence of multiple sclerosis in the region of Osona, Catalonia, northern Spain. *J Neurol Neurosurg Psychiatry* 1995;58:577-81.
17. Wynn DR, Rodriguez M, O'Fallon WM, Kurland LT. A reappraisal of the epidemiology of multiple sclerosis in Olmsted County, Minnesota. *Neurology* 1990;40:780-6.
18. Svenningsson A, Runmarker B, Lycke J, Andersen O. Incidence of MS during two fifteen-year periods in the Gothenburg region of Sweden. *Acta Neurol Scand* 1990;82:161-8.
19. Cook SD, Cromarty JJ, Tapp W, Poskanzer D, Walker JD, Dowling PC. Declining incidence of multiple sclerosis in the Orkney Islands. *Neurology* 1985;35:545-51.
20. Midgard R, Riise T, Nyland H. Epidemiologic trends in multiple sclerosis in More and Romsdal, Norway: a prevalence/incidence study in a stable population. *Neurology* 1991;41:887-92.
21. Kurtzke JF, Hyllested K. Multiple sclerosis in the Faroe Islands. III. An alternative assessment of the three epidemics. *Acta Neurol Scand* 1987;76:317-39.
22. Alter M, Leibowitz U, Speer J. Risk of multiple sclerosis related to age at immigration to Israel. *Arch Neurol* 1966;15:234-7.
23. Dean G. Annual incidence, prevalence, and mortality of multiple sclerosis in white South-African-born and in white immigrants to South Africa. *BMJ* 1967;2:724-30.
24. Elian M, Nightingale S, Dean G. Multiple sclerosis among United Kingdom-born children of immigrants from the Indian subcontinent, Africa and the West Indies. *J Neurol Neurosurg Psychiatry* 1990;53:906-11.
25. Compston A. Risk factors for multiple sclerosis: race or place? *J Neurol Neurosurg Psychiatry* 1990;53:821-3.
26. Ebers GC, Sadovnick AD, Risch NJ, Canadian Collaborative Study Group. A genetic basis for familial aggregation in multiple sclerosis. *Nature* 1995;377:150-1.
27. Sadovnick AD, Armstrong H, Rice GP, et al. A population-based study of multiple sclerosis in twins: update. *Ann Neurol* 1993;33:281-5.
28. Sadovnick AD, Baird PA, Ward RH. Multiple sclerosis: updated risks for relatives. *Am J Med Genet* 1988;29:533-41.
29. Jersild C, Fog T, Hansen GS, Thomsen M, Svejgaard A, Dupont B. Histocompatibility determinants in multiple sclerosis, with special reference to clinical course. *Lancet* 1973;2:1221-5.
30. Marrosu MG, Murru MR, Costa G, Murru R, Muntoni F, Cucca F. DRB1-DQA1-DQB1 loci and multiple sclerosis predisposition in the Sardinian population. *Hum Mol Genet* 1998;7:1235-7.
31. Sawcer S, Jones HB, Feakes R, et al. A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. *Nat Genet* 1996;13:464-8.
32. Ebers GC, Kukay K, Bulman DE, et al. A full genome search in multiple sclerosis. *Nat Genet* 1996;13:472-6.
33. Haines JL, Ter-Minassian M, Bazyk A, et al. A complete genomic screen for multiple sclerosis underscores a role for the major histocompatibility complex. *Nat Genet* 1996;13:469-71.
34. Kuokkanen S, Gschwend M, Rioux JD, et al. Genomewide scan of multiple sclerosis in Finnish multiplex families. *Am J Hum Genet* 1997;61:1379-87.
35. Haines JL, Terwedow HA, Burgess K, et al. Linkage of the MHC to familial multiple sclerosis suggests genetic heterogeneity. *Hum Mol Genet* 1998;7:1229-34.
36. Chataway J, Feakes R, Coraddu F, et al. The genetics of multiple sclerosis: principles, background and updated results of the United Kingdom systematic genome screen. *Brain* 1998;121:1869-87.
37. te Meerman GJ, Van der Meulen MA. Genomic sharing surrounding alleles identical by descent: effects of genetic drift and population growth. *Genet Epidemiol* 1997;14:1125-30.
38. Robertson NP, Compston DAS. Prognosis in multiple sclerosis: genetic factors. In: Siva A, Kesselring J, Thompson AJ, eds. *Frontiers in multiple sclerosis*. Vol. 2. London: Martin Dunitz, 1999:51-61.
39. Weinschenker BG, Santrach P, Bissonet AS, et al. Major histocompatibility complex class II alleles and the course and outcome of MS: a population-based study. *Neurology* 1998;51:742-7.
40. Schrijver HM, Crusius JB, Uitdehaag BM, et al. Association of interleukin-1beta and interleukin-1 receptor antagonist genes with disease severity in MS. *Neurology* 1999;52:595-9.
41. Myhr KM, Raknes G, Nyland H, Vedeler C. Immunoglobulin G Fc-receptor (Fc gamma R) IIA and IIB polymorphisms related to disability in MS. *Neurology* 1999;52:1771-6.
42. Evangelou N, Jackson M, Beeson D, Palace J. Association of the APOE epsilon4 allele with disease activity in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1999;67:203-5.
43. Hohlfeld R. Biotechnological agents for the immunotherapy of multiple sclerosis: principles, problems and perspectives. *Brain* 1997;120:865-916.
44. Lassmann H, Raine CS, Antel J, Prineas JW. Immunopathology of multiple sclerosis: report on an international meeting held at the Institute of Neurology of the University of Vienna. *J Neuroimmunol* 1998;86:213-7.
45. Wolswijk G. Chronic stage multiple sclerosis lesions contain a relatively quiescent population of oligodendrocyte precursor cells. *J Neurosci* 1998;18:601-9.
46. Waxman SG. Demyelinating diseases — new pathological insights, new therapeutic targets. *N Engl J Med* 1998;338:323-5.
47. Rivera-Quinones C, McGavern D, Schmelzer JD, Hunter SF, Low PA, Rodriguez M. Absence of neurological deficits following extensive demyelination in a class I-deficient murine model of multiple sclerosis. *Nat Med* 1998;4:187-93.
48. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998;338:278-85.
49. Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. A quantitative analysis of oligodendrocytes in multiple sclerosis lesions: a study of 113 cases. *Brain* 1999;122:2279-95.
50. Bitsch A, Schuchardt J, Bunkowski S, Kuhlmann T, Brück W. Acute axonal injury in multiple sclerosis: correlation with demyelination and inflammation. *Brain* 2000;123:1174-83.
51. Schmidt S, Linington C, Zipp F, et al. Multiple sclerosis: comparison of the human T-cell response to S100beta and myelin basic protein reveals parallels to rat experimental autoimmune panencephalitis. *Brain* 1997;120:1437-45.
52. van Noort JM, van Sechel AC, Bajramovic JJ, et al. The small heat-shock protein alphaB-crystallin as candidate autoantigen in multiple sclerosis. *Nature* 1996;375:798-801.
53. Steinman L. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell* 1996;85:299-302.
54. Lucchinetti CE, Bruck W, Rodriguez M, Lassmann H. Multiple sclerosis: lessons from neuropathology. *Semin Neurol* 1998;18:337-49.
55. Piddlesden SJ, Lassmann H, Zimprich F, Morgan BP, Linington C. The demyelinating potential of antibodies to myelin oligodendrocyte glycoprotein is related to their ability to fix complement. *Am J Pathol* 1993;143:555-64.
56. Linington C, Bradl M, Lassmann H, Brunner C, Vass K. Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. *Am J Pathol* 1988;130:443-54.
57. Raine CS, Cannella B, Hauser SL, Genain CP. Demyelination in primate autoimmune encephalomyelitis and acute multiple sclerosis lesions: a case for antigen-specific antibody mediation. *Ann Neurol* 1999;46:144-60.
58. Storch MK, Piddlesden S, Haltia M, Iivanainen M, Morgan P, Lassmann H. Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination. *Ann Neurol* 1998;43:465-71.
59. Carrigan DR, Harrington D, Knox KK. Subacute leukoencephalitis caused by CNS infection with human herpesvirus-6 manifesting as acute multiple sclerosis. *Neurology* 1996;47:145-8.
60. Challoner PB, Smith KT, Parker JD, et al. Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. *Proc Natl Acad Sci U S A* 1995;92:7440-4.
61. Sriram S, Stratton CW, Yao S, et al. Chlamydia pneumoniae infection of the central nervous system in multiple sclerosis. *Ann Neurol* 1999;46:6-14.
62. Boman J, Roblin PM, Sundstrom P, Sandstrom M, Hammerschlag MR. Failure to detect Chlamydia pneumoniae in the central nervous system of patients with MS. *Neurology* 2000;54:265.
63. Lucchinetti CE, Bruck W, Rodriguez M, Lassmann H. Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. *Brain Pathol* 1996;6:259-74.
64. Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* 2000;47:707-17.
65. Paty DW, Li DK, UBC MS/MRI Study Group, IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology* 1993;43:662-7.
66. The IFNB Multiple Sclerosis Study Group, University of British Columbia MS/MRI Analysis Group. Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. *Neurology* 1995;45:1277-85.
67. Simon JH, Jacobs LD, Campion M, et al. Magnetic resonance studies

- of intramuscular interferon β -1a for relapsing multiple sclerosis. *Neurology* 1998;43:79-87.
- 68.** PRISMS (Prevention of Relapses and Disability by Interferon β -1a Subcutaneously in Multiple Sclerosis) Study Group. Randomised double-blind placebo-controlled study of interferon β -1a in relapsing/remitting multiple sclerosis. *Lancet* 1998;352:1498-504. [Erratum, *Lancet* 1999; 353:678.]
- 69.** Miller DH, Molyneux PD, Barker GJ, MacManus DG, Moseley IF, Wagner K. Effect of interferon β -1b on magnetic resonance imaging outcomes in secondary progressive multiple sclerosis: results of a European multicenter, randomized, double-blind, placebo-controlled trial. *Ann Neurol* 1999;46:850-9.
- 70.** Filippi M, Rocca MA, Martino G, Horsfield MA, Comi G. Magnetization transfer changes in the normal appearing white matter precede the appearance of enhancing lesions in patients with multiple sclerosis. *Ann Neurol* 1998;43:809-14.
- 71.** Allen IV, McKeown SR. A histological, histochemical and biochemical study of the macroscopically normal white matter in multiple sclerosis. *J Neurol Sci* 1979;41:81-91.
- 72.** Goodkin DE, Rooney WD, Sloan R, et al. A serial study of new MS lesions and the white matter from which they arise. *Neurology* 1998;51: 1689-97.
- 73.** Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T, Pregnancy in Multiple Sclerosis Group. Rate of pregnancy-related relapse in multiple sclerosis. *N Engl J Med* 1998;339:285-91.
- 74.** Sibley WA, Bamford CR, Clark K. Clinical viral infections and multiple sclerosis. *Lancet* 1985;1:1313-5.
- 75.** Miller AE, Morgante LA, Buchwald LY, et al. A multicenter, randomized, double-blind, placebo-controlled trial of influenza immunization in multiple sclerosis. *Neurology* 1997;48:312-4.
- 76.** European Study Group on Interferon β -1b in Secondary Progressive MS. Placebo-controlled multicentre randomised trial of interferon β -1b in treatment of secondary progressive multiple sclerosis. *Lancet* 1998;352: 1491-7.
- 77.** Goodkin DE, North American Study Group on Interferon beta-1b in Secondary Prevention MS. Interferon beta-1b in secondary progressive MS: clinical and MRI results of a 3-year randomized controlled trial. *Neurology* 2000;54:Suppl:2352. abstract.
- 78.** Tubridy N, Behan PO, Capildeo R, et al. The effect of anti-alpha4 integrin antibody on brain lesion activity in multiple sclerosis. *Neurology* 1999;53:466-72.
- 79.** Beck RW, Cleary PA, Anderson MM Jr, et al. A randomized, controlled trial of corticosteroids in the treatment of acute optic neuritis. *N Engl J Med* 1992;326:581-8.
- 80.** Beck RW. The Optic Neuritis Treatment Trial: three-year follow-up results. *Arch Ophthalmol* 1995;113:136-7.
- 81.** Weinschenker BG, O'Brien PC, Petterson TM, et al. A randomized trial of plasma exchange in acute central nervous system inflammatory demyelinating disease. *Ann Neurol* 1999;46:878-86.
- 82.** Comi G, Filippi M, Barkhof F, et al. Interferon beta 1a (Rebif) in patients with acute neurological syndromes suggestive of multiple sclerosis: a multi-center, randomized, double-blind, placebo-controlled study. *Neurology* 2000;54:Suppl 3:A85-A86. abstract.
- 83.** Jacobs LD, Beck RW, Simon JH, et al. Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. *N Engl J Med* 2000;343:898-904.
- 84.** The IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology* 1993;43:655-61.
- 85.** Johnson KP, Brooks BR, Cohen JA, et al. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. *Neurology* 1995;45:1268-76.
- 86.** Johnson KP, Brooks BR, Cohen JA, et al. Extended use of glatiramer acetate (Copaxone) is well tolerated and maintains its clinical effect on multiple sclerosis relapse rate and degree of disability. *Neurology* 1998;50:701-8.
- 87.** Jacobs LD, Cookfair DL, Rudick RA, et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. *Ann Neurol* 1996;39:285-94. [Erratum, *Ann Neurol* 1996;40:480.]
- 88.** Rudick RA, Goodkin DE, Jacobs LD, et al. Impact of interferon beta-1a on neurologic disability in relapsing multiple sclerosis. *Neurology* 1997; 49:358-63.
- 89.** Forbes RB, Lees A, Waugh N, Swingler RJ. Population based cost utility study of interferon beta-1b in secondary progressive multiple sclerosis. *BMJ* 1999;319:1529-33.
- 90.** van den Noort S, Eidelman B, Rammohan K, et al. National Multiple Sclerosis Society (NMSS): disease management consensus statement. New York: National MS Society, 1998.
- 91.** Rodriguez M, Siva A, Ward J, Stolp-Smith K, O'Brien P, Kurland L. Impairment, disability, and handicap in multiple sclerosis: a population-based study in Olmsted County, Minnesota. *Neurology* 1994;44:28-33.
- 92.** The Once Weekly Interferon for MS Study Group. Evidence of interferon beta-1a dose response in relapsing-remitting MS. *Neurology* 1999; 53:679-86.
- 93.** Freedman MS, PRISMS Study Group. PRISMS 4-year results: evidence of clinical dose effect of interferon beta-1a in relapsing MS. *Neurology* 2000;54:Suppl:2351. abstract.
- 94.** Fazekas F, Deisenhammer F, Strasser-Fuchs S, Nahler G, Mamoli B. Randomised placebo-controlled trial of monthly intravenous immunoglobulin therapy in relapsing-remitting multiple sclerosis. *Lancet* 1997;349: 589-93.
- 95.** Paty D. Results of the 3-year, double-blind, placebo-controlled study of interferon beta-1a (Rebif) in secondary-progressive MS. Presented at the Ninth Annual Meeting of the European Neurological Society, Milan, Italy, June 5-9, 1999.
- 96.** Hughes R, SPECTRIMS Group. "Relapsing" versus "nonrelapsing" SPMS: different prognosis and response to interferon therapy in the SPECTRIMS study. *Neurology* 2000;54:Suppl 3:A233. abstract.
- 97.** Krapf H, Morrissey SP, Zenker O, et al. Mitoxantrone in progressive multiple sclerosis: MRI results of the European phase III trial. *Neurology* 1999;52:Suppl 2:A495. abstract.
- 98.** Rudge P. Are clinical trials of therapeutic agents for MS long enough? *Lancet* 1999;353:1033-4.
- 99.** Noseworthy JH, Gold R, Hartung H-P. Treatment of multiple sclerosis: recent trials and future perspectives. *Curr Opin Neurol* 1999;12:279-93.
- 100.** van Oosten BW, Barkhof F, Truyen L, et al. Increased MRI activity and immune activation in two multiple sclerosis patients treated with the monoclonal anti-tumor necrosis factor antibody cA2. *Neurology* 1996;47: 1531-4.
- 101.** The Lenercept Multiple Sclerosis Study Group, University of British Columbia MS/MRI Analysis Group. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. *Neurology* 1999;53: 457-65.
- 102.** Compston A. Remyelination in multiple sclerosis: a challenge for therapy: the 1996 European Charcot Foundation Lecture. *Mult Scler* 1997;3:51-70.
- 103.** Archer DR, Cuddeon PA, Lipsitz D, Duncan LD. Myelination of the canine central nervous system by glial cell transplantation: a model for repair of human myelin disease. *Nat Med* 1997;3:54-9.
- 104.** Noseworthy JH, O'Brien P, Erickson BJ, et al. The Mayo Clinic-Canadian Cooperative Trial of sulfasalazine in active multiple sclerosis. *Neurology* 1998;51:1342-52.