MEDICAL PROGRESS

MULTIPLE SCLEROSIS
(First of Two Parts)

DALE E. McFARLIN, M.D., AND HENRY F. McFARLAND, M.D.

The lesions of multiple sclerosis were probably described as early as 1838, but it was Charcot in 1869 who recognized the characteristic clinical and pathologic features of the disease. Since that time, the disorder has been studied extensively. Numerous distinguished, even renowned physicians and scientists have investigated various aspects of multiple sclerosis. Although much has been learned and many theories have emerged, the cause and pathogenesis of this disease remains unknown. No preventive measures or definitive therapies exist. The purpose of this article is to review the classic clinical and pathologic aspects of the disease and recent scientific findings that may provide insights into the underlying pathogenic mechanisms.

Clinically, multiple sclerosis is highly variable, and there is no specific diagnostic test. This variability is a source of frustration for patients and their families, as well as for physicians, because it confuses the diagnosis and hampers both the investigation of possible causative mechanisms and the design and interpretation of therapeutic trials. The disease usually begins between the second and fifth decade, but both earlier and later ages of onset have been reported. Sensory, visual, and motor dysfunction are common in patients with multiple sclerosis. In approximately 60% of patients the disease is initially manifested by exacerbations and remissions. In the early stages of disease the remissions are usually associated with complete or nearly complete return of normal neurologic function, but with each remission there is less improvement and greater neurologic dysfunction. The disease enters a chronic phase and becomes progressively worse over the years.

Other clinical courses that may be seen have been described as acute, progressive, and benign. In the acute form of multiple sclerosis neurologic dysfunction progresses rapidly over a few weeks or months. The course is either monophasic or manifested by incomplete remissions of short duration, followed by severe relapses. The process can be terminal within a few months.

In patients with the progressive form, neurologic dysfunction gradually becomes worse after the onset of the disease, without well-delineated remissions and relapses. Chronic progressive paraplegia is an example of this form. In such patients dissemination of lesions may be difficult to document clinically, but electrophysiologic evaluation often shows lesions in the visual, auditory, or somatic sensory pathways and thus can aid in the diagnosis. The rate of progression varies considerably from one patient to another.

Unlike these relatively severe forms of disease, the benign form is characterized by only a few exacerbations—often mild—followed by complete recovery. These patients remain relatively asymptomatic and have no marked impairment of function for many years. The possibility of this benign course provides the basis for guarded optimism in patients who have recently been diagnosed, and is one reason that physicians are reluctant to use life-threatening experimental therapy early in the disease.

Finally, it seems likely that a subclinical form of the disease can occur, because demyelination is occasionally found at autopsy in asymptomatic patients.

In a condition that has such a variety of clinical presentations, it is logical to question whether more than one disease process is operative. The reason for believing that all the various clinical syndromes are closely related is that common pathologic features are found, regardless of the clinical course.

Pathology

The primary pathology is confined to the nervous system, where macroscopic lesions ranging from 1 mm to 4 cm are scattered throughout the white matter. These are known as plaques. There are distinct regions of predilection, such as the periventricular areas, and the lesions tend to be symmetrical. The color of individual plaques correlates with their age. New lesions are pink, whereas older ones are gray. The terms "multiple sclerosis" and "disseminated sclerosis" were initially used to describe the wide distribution of discrete lesions of various ages in the white matter. Microscopically, the characteristic feature is the breakdown of the myelin sheath, with relative sparing of axons. Although Wallerian degeneration can be observed, particularly in advanced phases of the disease or in the severe lesions of the acute form, this abnormality is disproportional to the striking destruction of myelin. The demyelinating lesions have a perivenous distribution and contain macrophages, lymphocytes, and plasma cells. In new lesions products from the breakdown of myelin, largely lipids, are found free and in macrophages. In time they become less prominent, and astrocytic hyperplasia occurs.

Microscopical analysis of multiple-sclerosis plaques
has led to the conclusion that oligodendroglia, the myelin-producing cells, are reduced in number and frequently absent, at least in relatively old lesions. In formulating a concept of pathogenesis, major consideration has been given to the possibility that multiple sclerosis is a disease of the oligodendroglia. It is relevant that in experimental diseases primarily affecting oligodendroglia, as well as in most cases of multiple sclerosis, loss of myelin occurs throughout each affected node of Ranvier. However, in some multiple-sclerosis lesions loss of myelin does not follow this pattern and is uneven in a given node. In addition, a recent ultrastructural analysis of a single lesion identified oligodendroglia in areas of acute demyelination. Such observations suggest that the primary site of the pathology is the myelin membrane rather than the oligodendroglia. Myelin is the most distal portion of the oligodendroglialcyte membrane, and at the current level of understanding, it is important to distinguish the pathologic processes that involve the oligodendroglialcyte cell body from those that selectively affect myelin. For example, injection of an oligodendroglialcyte would be expected to result in the destruction of all myelin produced by that cell, but an immune reaction against myelin might be unrelated to individual oligodendroglia.

Multiple-sclerosis lesions contain small amounts of myelin basic protein and increased amounts of proteolytic enzymes, but the sequence of myelin breakdown is not well established. Immunocytochemical studies have shown a loss of the myelin basic protein and one of the myelin-associated glycoproteins in areas of myelin breakdown. In addition, immunologic staining has shown that the region of decreased myelin-associated glycoprotein extends far beyond the margin of demyelination. This finding is of considerable interest and suggests that the myelin-associated glycoprotein is altered before destruction of the myelin occurs. In animal studies myelin-associated glycoprotein has been identified in developing oligodendroglia and myelin sheaths, but in mature myelin it is located periaxionally, where oligodendrogial membranes surround axons. In multiple sclerosis the alterations in periaxial myelin-associated glycoprotein, shown by immunocytochemical staining, have been interpreted as abnormalities of the oligodendroglia that precede myelin breakdown. The distributions of myelin-associated glycoprotein and myelin basic protein were not analyzed in studies in which ultrastructural findings suggested that the disease affects the myelin membrane rather than the oligodendroglia. Further investigations of myelin glycoproteins in multiple-sclerosis lesions of different ages and in experimental diseases known to affect oligodendroglia should provide useful information.

**Diagnosis**

Criteria for the clinical diagnosis of multiple sclerosis have been developed. A definite diagnosis of the disease requires documentation of lesions that have occurred on more than one occasion and at more than one site, and that are not explained by other mechanisms. The presence of lesions has traditionally been documented on the basis of clinical findings, but in recent years electrophysiologic evaluations and computerized tomography have aided the identification of clinically silent lesions in some patients. It is now common to use these procedures as extensions of the neurologic examination.

**Laboratory Studies**

**Electrophysiologic Studies**

Investigation of the visual, auditory, and somatic sensory pathways can provide evidence of lesions in the respective regions of the nervous system. It is important to note that these techniques simply demonstrate the presence of lesions but provide no information concerning the cause. It is unnecessary to perform such studies on patients in whom the diagnosis of multiple sclerosis can be established clinically, because these procedures do not affect routine treatment, and they are time-consuming and expensive. However, they may be useful in evaluating patients receiving experimental therapeutic agents.

**Computerized Tomography**

Routine x-ray studies of patients with multiple sclerosis are normal. Lesions can be identified in the white matter by means of computerized tomography, particularly when combined with contrast enhancement. It is not necessary to study every patient in this manner, but computerized tomography may help provide evidence of multiple lesions in some patients. We have found this approach particularly useful in documenting acute forms of the disease, with abnormalities of cognitive function. Nuclear-magnetic-resonance imaging has recently been used to evaluate multiple sclerosis, and in one study, it was used to identify lesions as small as 3 by 4 mm. To date there has been no pathological confirmation that such lesions represent multiple-sclerosis plaques, but if such confirmation can be obtained, nuclear-magnetic-resonance imaging may become a valuable diagnostic aid.

**Cerebrospinal Fluid**

Although there is no laboratory test that can be used to diagnose multiple sclerosis, changes in the cerebrospinal fluid can be helpful. A mild increase in protein or lymphocytes (or both) may be seen, and in the majority of patients, the level of IgG in the cerebrospinal fluid is elevated. Immunglobulins in the cerebrospinal fluid are present in much lower quantities than in the serum. Trace amounts of blood in the cerebrospinal fluid can lead to spurious increases in cerebrospinal-fluid immunoglobulins. Thus, to exclude the possibility of a breakdown in the blood-brain barrier, it is imperative to express cerebrospinal-fluid immunoglobulins as ratios to total protein or to albumin. In multiple sclerosis IgG is synthesized with-
in the central nervous system, and approaches have been developed to quantify this process. Also, the cerebrospinal-fluid IgG in multiple sclerosis is relatively homogeneous in charge; after electrophoresis it is distributed in a small number of discrete (oligoclomal) bands. The oligoclonal banding in the cerebrospinal fluid of a given patient with multiple sclerosis remains relatively constant throughout the duration of the disease. Although the findings vary among different series, approximately 70 per cent of patients with multiple sclerosis have elevated cerebrospinal-fluid IgG, and approximately 90 per cent have oligoclonal bands.

Levels of cerebrospinal-fluid IgM and IgA may also be elevated. In the proper clinical setting detection of immunoglobulin abnormalities may aid in establishing a diagnosis of multiple sclerosis; however, they are not pathognomonic for the disease and can be seen in other disorders. It should be noted that a few patients with definite multiple sclerosis, defined clinically, have normal cerebrospinal-fluid immunoglobulins and lack oligoclonal bands.

Fragments of myelin have been observed in the cerebrospinal fluid of patients with multiple sclerosis, and it has been shown that myelin basic protein or a proteolytic fragment of myelin basic protein in the cerebrospinal fluid is transiently increased after acute episodes. Analysis of cerebrospinal fluid obtained within one week of an acute clinical episode shows an elevated level of myelin basic protein or of a proteolytic fragment of myelin basic protein in approximately 90 per cent of patients. Over the next two weeks these levels return to normal in the majority of patients. Those with chronic forms of the disease tend to have a normal level of myelin basic protein in their cerebrospinal fluid. A minor fluctuation in the level of myelin basic protein in these patients may reflect changes in disease activity, but this will require further study. Fever is known to accentuate the symptoms of multiple sclerosis, probably because defects in axon conduction become more pronounced as body temperature increases. In patients with fever the proteolytic fragment of myelin basic protein in the cerebrospinal fluid remains normal. Elevated levels of myelin basic protein or of the proteolytic fragment of myelin basic protein have also been found in patients with infarction of the central nervous system, encephalitis, leukodystrophies, metabolicencephalopathies, or methotrexate myelopathy, and the elevations probably reflect tissue destruction. Thus, elevated levels of myelin basic protein or of the proteolytic fragment of myelin basic protein in the spinal fluid are laboratory indicators of active demyelination. It is likely that other components of myelin or proteolytic enzymes are also increased in the cerebrospinal fluid or in the blood; these could be useful markers of disease activity.

Blood Leukocytes

In recent years various methods have been used to evaluate peripheral blood leukocytes. Collectively, the results are conflicting. Several factors contribute to the lack of consensus. Investigators have employed different methods, used different criteria for defining normality, and in many situations studied patients at different stages of the disease. Some investigators have ignored the possible role of therapeutic agents, such as steroids, in altering leukocyte distribution. The control groups of patients have frequently been inadequate, and few longitudinal studies have followed patients with well-defined forms of the disease. Many assessments have been made on the basis of in vitro phenomena, and because of the perplexing nature of multiple sclerosis, there has been a tendency to emphasize minor abnormalities in an effort to identify clues to the underlying problem. In spite of these difficulties, some common findings have emerged in recent years.

Variations in the distribution of T and B cells, as well as changes in lymphocyte subpopulations, have been documented, particularly during acute exacerbations. Although the number of T cells remains unchanged during the long-term course of multiple sclerosis, at the time of acute exacerbation a slight fall in the total number of T cells has been shown by the use of both sheep-cell rosetting techniques and monoclonal antibodies. Of particular interest are recent studies that have used various methods to document changes in lymphocyte subpopulations. Variations in T-gamma cells, the lymphocyte subset that binds the Fc portion of IgG, have been observed. In some studies these subpopulations were found to be increased, but in others they were reduced. The differences in these findings may be related to disease activity, and the findings from longitudinal investigations support this explanation. In two patients the T-gamma population diminished during acute attacks, returned to normal at the time of clinical improvement, and subsequently increased above normal.

Perhaps the most compelling data come from studies in which monoclonal antibodies were used as phenotypic markers of lymphocyte subsets. OKT3 was generally used as a marker for the total number of T cells, OKT4 as a marker for a helper or inducer subpopulation, and OKT5 or OKT8 as a marker for a suppressor/cytotoxic subpopulation. Not only was the total number of T cells reduced during acute exacerbation, as shown by the OKT3 marker, but there was a specific reduction in the number of cells identified by the OKT5 marker. A subsequent longitudinal study of four patients showed some shifts in T-cell subsets, which were partially correlated with disease activity. For example, in one clinically stable patient, no changes were seen in multiple determinations. In contrast, in another patient with an active clinical disease, abnormal T-cell ratios were documented in 12 of 27 assays performed during a six-month period. It is noteworthy that when a different series of lymphocyte markers was used to analyze patients in western Canada, no changes were found during acute attacks; however, patients with a chronic
progressive form of multiple sclerosis had reduced lymphocyte subpopulations bearing the Leu 2a marker, which probably identifies the same T-cell subset that is identified by OKT5 and OKT8.

Functional changes in peripheral-blood T cells have also been observed. A mild reduction in response to mitogens, particularly during the acute phase, has been reported by some but not all investigators. The capacity of concanavalin A-stimulated lymphocytes to induce suppression of a second mitogenic response by peripheral-blood leukocytes is reduced during acute episodes. This concanavalin A-induced suppressor activity returns to normal after exacerbation and is also normal in patients with chronic or stable disease. However, some patients with chronic disease lack normal suppressor activity in response to neuroantigens and measles virus. These findings have led to the hypothesis that reduced T-cell suppressor function may permit an immunologic response that leads to myelin damage.

It has been suggested that the lymphocyte-suppressor activity of concanavalin A-activated cells is mediated through interferon. Lymphocytes from approximately 33 per cent of patients showed a decrease in the production of interferon in response to viruses and other inducers of interferon. In one study reduced interferon production was paralleled by impaired natural killer activity, but natural killer activity has been assessed in other laboratories, and there is no consensus concerning the findings in multiple sclerosis. To measure this activity, peripheral-blood leukocytes and target cells are incubated at various ratios. Target cells consist of transformed or virus-infected cell lines. In one study the demonstration of quantitative differences in natural killer activity between patients with multiple sclerosis and controls required high killer-to-target ratios and depended on the type of target cells employed. Other investigators using different target cells have observed no abnormalities in natural killer activity.

The blood from patients with acute and chronic forms of multiple sclerosis has recently been shown to have a marked increase in antibody-dependent cellular cytotoxicity. This activity is believed to be mediated by a subset of T cells with receptors for the Fc portions of IgM and IgG. They have been designated T-mu-gamma cells. Peripheral-blood leukocytes from patients with multiple sclerosis have been reported to have altered surface properties and a tendency to adhere to brain sections, myelin antigens, erythrocytes, and other cells. Similar phenomena have been described in other chronic diseases and brain tumors. The functional importance of these reactions is not known.

Although considerable effort has been focused on the blood T cells and natural killer cells, many patients have increased numbers of Ia-positive cells. These could represent activated T cells, but there are other Ia-positive cells that may be important. For example, B cells are Ia positive, and some data indicate that blood B cells in patients with multiple sclerosis are less responsive to suppressor signals than they are in the absence of disease. Such findings could explain abnormalities of immunoglobulins without implicating a T-cell lesion. A second Ia-positive cell population is the macrophage; in our view investigation of these cells has been relatively neglected. Macrophages are recruited by T cells in cell-mediated immune reactions, and increased numbers of such cells in the blood could be expected in a process associated with T-cell activation. Furthermore, macrophages are prominent in the cellular reaction in the nervous system and contain several proteolytic enzymes that could contribute to myelin destruction. A better understanding of the role of macrophages may have therapeutic importance because of the potential for pharmacologic modification of enzyme release or activity, which could reduce tissue damage.

The cause of abnormalities in blood lymphocytes is not known, but three possibilities have been considered. Alterations may result from changes in leukocyte migration that are associated with acute clinical episodes. OKT3 and OKT5 identify subpopulations of T lymphocytes with both cytotoxic and suppressor functions. The possibility that multiple sclerosis is related to a cell-mediated immunopathologic process has led to speculation that these cells are sequestered in the central nervous system. No increase in OKT8 subpopulations of lymphocytes was found in the cerebrospinal fluid; however, this finding does not exclude a relative increase in the number of cells in lesions of the central nervous system. Reduction of the suppressor/cytotoxic subset could result from the same pathologic mechanisms that cause the lesions. As discussed below, some findings indicate that multiple sclerosis may be the consequence of a chronic infection, and it is possible that a subset of lymphocytes and oligodendroglia are receptive to the same infectious agent. An autoimmune basis for multiple sclerosis has also been postulated, and autoantibodies against lymphocytes have been observed in some patients, although they have not been shown to correlate with either lymphocyte abnormalities or longitudinal disease activity. It is also possible that autoantibodies against the T5/T8 molecule modulate or block this antigen. Furthermore, it has been proposed that there is cross-reactivity between antigens in myelin and the T5/T8 subpopulation of blood lymphocytes. Thus, the changes in peripheral-blood leukocytes may contribute to the pathogenesis or may simply be phenomena.

It is important to emphasize that the abnormalities in peripheral-blood leukocytes represent relatively minor deviations from normal findings. Longitudinal studies have shown that many patients with multiple sclerosis enter a chronic progressive phase of the disease and slowly decline. This leads to the conclusion that there is subtle but active disease in the nervous system that is not mirrored by clear clinical changes. If the disease process is immunopathologically mediat-
ed, it is possible that only minimal effects, if any, will be reflected outside the central nervous system. Since many of the concepts of immune regulation in laboratory animals have been derived from studies of cells in lymphoid organs, rather than blood cells, perhaps investigation of lymphoid structures in multiple sclerosis would provide more convincing information about the possibility of an aberrant immune response. Unfortunately, good approaches to the investigation of the immune response within the human nervous system do not exist. New insights may emerge from the study of experimental models of disease. It is also possible that some forms of experimental therapy now being tried both in animal models and in patients with multiple sclerosis will provide clues to the nature of the immune process within the nervous system.

Association with the Major Histocompatibility Complex

Like many other diseases, multiple sclerosis is associated with particular antigens of the major histocompatibility complex. In whites from northern Europe, North America, and Australia the disease is linked to HLA-A3 and B-7, and the strongest association in these populations is with Dw2 and DRw2. In Israel and northern Italy an association with HLA-A-3 and B-7 has not been found, but analysis of DRw antigens in northern Italy and Jordan has demonstrated a strong correlation with DRw4, and a possible association with DRw6 has been found in Japan. These findings suggest that the disease may be linked to different genetic backgrounds. At the most recent international histocompatibility workshop, it was concluded that the risk in Italian patients is associated with DRw2, and a strong association between HLA antigens and the disease in Japanese or Israeli patients was not documented. However, the size of these groups was relatively small, and further analysis of the association between multiple sclerosis and human leukocyte antigens in various ethnic groups is appropriate.

The relevance of the correlation between multiple sclerosis and HLA antigens is not known. Because the strongest association is with antigens specified by the DRw region, a relation between susceptibility to disease and genetic control of immune regulation has been postulated. Because of the increased frequency of these HLA antigens in multiple sclerosis, considerable attention has been directed at identifying the possible genetic influence on susceptibility to disease. It has long been recognized that the risk of multiple sclerosis is slightly increased in first-degree and second-degree relatives of affected patients. Attempts to identify a genetic linkage have focused primarily on studies of families in which more than one member was involved, in the hope of demonstrating a haplotype or gene product that was shared by affected members and possibly absent in unaffected members. To date these studies have failed to identify an absolute correlation between disease and HLA haplotype. Thus, a gene that increases susceptibility to multiple sclerosis has not been conclusively demonstrated. Possible subclinical disease, inaccurate diagnosis, and failure to identify the critical genetic markers were sources of potential error in these family studies.

A complementary method of evaluating the genetic influences in multiple sclerosis has been the investigation of twins. In a study of 30 sets of twins in which both members of each pair were the same sex, higher concordance was found in monozygotic twins than in dizygotic twins; however, only 50 per cent of the former were concordant. In addition, two sets of dizygotic twins were concordant, and in neither of these sets were the members haplotype with respect to HLAs. In view of these findings, it seems unlikely that the increased incidence of multiple sclerosis in families, and specifically in twins, is based solely on genetic influence. The common environment shared by twins and other family members cannot be ignored.

References
