

# 18

## Multiple Sclerosis and Other Demyelinating Diseases

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### 18.1. Introduction

Multiple sclerosis (MS) is the most common idiopathic demyelinating disease of the central nervous system (CNS). Although partially effective treatments are now available, MS represents a major target for research into the development of disease-modifying therapies that specifically focus on the neuroimmune pathways of myelin and tissue damage that currently are incompletely understood. Multiple sclerosis is considered to be an example of development of autoimmunity to self-antigens within the CNS through multiple initiating events that include infections and other environmental factors. The direct or indirect induction of immune responses against CNS antigens includes chemotaxis of T cells, B cells, and monocytes, and production of immunoglobulin responses, each of which can act as an effector of myelin damage that occurs in distinct histological patterns. Because a specific cause for MS has not been identified, much MS research has focused on CNS immune responses triggered by unidentified insults that in turn trigger inflammation-mediated cascades of myelin and cellular damage that are likely relevant to other neurodegenerative diseases. This chapter discusses current the epidemiology, etiology, pathophysiology, animal models, virus models and recent advances in the neuroimmunology of MS from the perspective of the potential for development of newer therapies for MS and other inflammatory CNS diseases.

### 18.2. Clinical Features and Diagnosis of Multiple Sclerosis

Multiple sclerosis (MS) is a disease of unknown etiology that primarily affects the myelin membrane and/or the oligodendroglia (myelin-producing cells) within the central nervous

system (CNS). The clinical features are highly variable, but they consistently reflect dysfunction due to inflammation, local edema, and destruction of the “central myelin” of the brain, spinal cord, and/or optic nerves (reviewed in Miller, 1996). Symptoms generally present with gradually increasing severity (days to weeks), followed by gradual resolution (weeks), which may be complete or partial. The frequency of different types of symptoms in MS is difficult to accurately establish, in part due to their highly variable severity and duration (Matthews, 1998). The most common neurological symptoms are focal sensory disturbances including numbness/tingling sensations, dysesthesiae (sensation), paresthesiae (abnormal sensation), L’hermitte’s sign (sudden electric-like sensations radiating into the arms or legs while flexing one’s neck), and occasionally burning pain. Such sensory disturbances occur in up to 70–80% of individuals during the course of MS (Miller, 1996; Matthews, 1998). Motor manifestations attributable to corticospinal tract dysfunction occur in up to 60% of patients, and often involve the lower extremities. Motor symptoms generally include dyscoordination and weakness that is often described as “heaviness” of an extremity, which may present unilaterally or bilaterally, in the case of spinal cord involvement. Optic nerve involvement, manifested as optic neuritis (inflammation of the optic nerve associated with visual loss) presents as the initial symptom in about 20% of MS patients, but its prevalence during the course of MS is thought to be much higher (Matthews, 1998).

The clinical course of the disease (reviewed in Ebers, 1998) is also variable, with approximately 70–85% of patients starting with a relapsing remitting disease pattern in which remissions are associated with complete or nearly complete recovery (Coyle, 2000). The disease typically gradually progresses (over years) so that remission periods are shorter and neurological recovery is incomplete. In the chronic phase, neurological dysfunction increases without significant improvement. Approximately 15% of patients have primary progressive MS, most commonly expressed as a progressive myelopathy. Other clinical courses have been described including an acute form with rapid neurologic deterioration and sometimes death within a few months, a progressive form

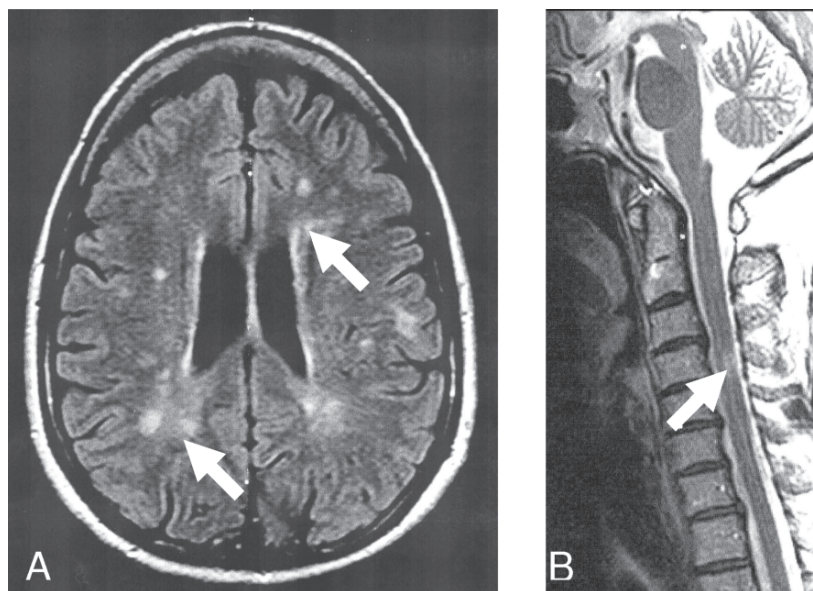


FIGURE 18.1. MRI signal abnormalities in the brain and spinal cord in a 55-year-old woman with multiple sclerosis. (A) FLAIR (Fluid attenuated inversion recovery) image of axial brain image at the level of the lateral ventricles. Arrows point to hyperintense periventricular lesions demonstrating a characteristic MS demyelination pattern; (B) T2W image of sagittal view of the spinal cord of the same individual, demonstrating focal bright signal (arrow) representing demyelination in the cervical spinal cord.

without defined remissions and relapses, and a benign form with a few exacerbations associated with complete recovery. A subclinical form has also been described based upon autopsy findings in asymptomatic individuals. MS can be viewed as a disease with multiple phenotypic presentations that are superimposed over pathological components that range from pure inflammation to gliosis.

Diagnostic criteria intended originally for the purposes of classifying patients for research protocols are now considered standard criteria for clinicians making the diagnosis of MS. The original clinical criteria of Schumacher (Schumacher et al., 1965) (reviewed in Coyle, 2000), were expanded by Poser (Poser et al., 1983) to incorporate paraclinical tests (MRI, cerebrospinal fluid analysis, and evoked potential testing) to increase the certainty of the diagnosis. The MRI typically demonstrates multifocal areas of demyelination surrounding the brain ventricles and within the spinal cord (Figure 18.1). The Poser criteria include the categories of clinically definite, laboratory-supported definite, probable, and possible multiple sclerosis. Previously, practicing clinicians generally attempted only to diagnose cases of definite MS (clinically definite or laboratory-supported definite) because of early recommendations for treatment of definite MS with the first FDA-approved immunomodulating medication, Interferon beta-1b/Betaseron. However, because recent clinical trials in individuals with single, clinically-apparent demyelinating events who did not meet Poser criteria for definite MS demonstrated that Interferon beta-1a (Avonex) greatly reduced the likelihood of development of definite MS over a 2-year period (Kinkel et al., 2006) clinicians have been advised to diagnose and treat patients with such isolated demyelinating events (Frohman et al., 2006b).

These diagnostic criteria, were again modified (Table 18.1) to include specific numbers and locations of MRI-defined lesions that can confirm the diagnosis of MS with a clinically monosymptomatic event or an insidious neurological progression suggestive of MS (McDonald et al., 2001; Polman et al., 2005). Included in Table 18.1 are the Poser criteria (Poser et al., 1983), McDonald criteria (McDonald et al., 2001) and the revised McDonald criteria (Polman et al., 2005). By the Poser criteria (Table 18.1, top two clinical presentations) “definite” multiple sclerosis requires objective evidence of central nervous system (CNS) dysfunction in  $\geq 2$  sites of involvement, predominantly in the white matter, relapsing-remitting or chronic progressive ( $>6$  months) in patients between the ages of 10 to 50 years at onset of symptoms. Importantly, there must be no better explanation of the symptoms. These “attacks” may be motor, sensory, visual, or coordination deficits and must last more than 24 h (typically days to weeks). They may be subjective and amnesic (i.e. recalled historically by the patient) or demonstrable by a physician, and separate attacks must be separated in time by at least 30 days of significant improvement to be classified as distinct attacks. In addition, if a physician can demonstrate objective dysfunction in two anatomically separate regions of the CNS, criteria are met for clinically definite multiple sclerosis, assuming no better explanations of the symptoms.

### 18.3. Epidemiology and Etiology of MS

MS is the most common inflammatory disease of the CNS, affecting an estimated 2,500,000 people worldwide and 350,000 in the United States alone (Johnson, 1994) (Kantarci

TABLE 18.1. Diagnostic criteria for multiple sclerosis (Polman et al., 2005).

Clinical presentation	Additional data needed for MS diagnosis
Two or more attacks <sup>a</sup> ; objective clinical evidence of two or more lesions	None <sup>b</sup>
Two or more attacks <sup>a</sup> ; objective clinical evidence of one lesion	Dissemination in space, demonstrated by: MRI <sup>c</sup> <i>or</i> Two or more MRI-detected lesions consistent with MS plus positive CSF <sup>d</sup> <i>or</i> Await further clinical attack <sup>a</sup> implicating a different site
One attack <sup>a</sup> ; objective clinical evidence of two or more lesions	Dissemination in time, demonstrated by: MRI <sup>c</sup> <i>or</i> Second clinical attack <sup>a</sup>
One attack <sup>a</sup> ; objective clinical evidence of one lesion (monosymptomatic presentation; clinically isolated syndrome)	Dissemination in space, demonstrated by MRI <sup>c</sup> <i>or</i>  Two or more MRI-detected lesions consistent with MS plus positive CSF <sup>d</sup> <i>and</i> Dissemination in time, demonstrated by: MRI <sup>c</sup> <i>or</i> Second clinical attack <sup>a</sup>
Insidious neurological progression suggestive of MS	One year of disease progression (retrospectively or prospectively determined) <i>and</i> Two of the following: a. Positive brain MRI (nine T2 lesions or four or more T2 lesions with positive VEP) <sup>f</sup> b. Positive spinal cord MRI (two focal T2 lesions) c. Positive CSF <sup>d</sup>

*Note:* If criteria indicated are fulfilled and there is no better explanation for the clinical presentation, the diagnosis is MS; if suspicious, but the criteria are not completely met, the diagnosis is “possible MS”; if another diagnosis arises during the evaluation that better explains the entire clinical presentation, then the diagnosis is “not MS.”

<sup>a</sup> An attack is defined as an episode of neurological disturbance for which causative lesions are likely to be inflammatory and demyelinating in nature. There should be subjective report (backed up by objective findings) or objective observation that the event lasts for at least 24 h.

<sup>b</sup> No additional tests are required; however, if tests (MRI, CSF) are undertaken and are negative, extreme caution needs to be taken before making a diagnosis of MS. Alternative diagnoses must be considered. There must be no better explanation for the clinical picture and some objective evidence to support a diagnosis of MS.

<sup>c</sup> MRI demonstration of space dissemination must fulfill the criteria derived from Barkhof and colleagues (Barkhof et al., 2003) and Tintore and coworkers (Tintore et al., 2003).

<sup>d</sup> Positive CSF determined by oligoclonal bands detected by established methods (isoelectric focusing) different from any such bands in serum or by an increased IgG index.

<sup>e</sup> MRI demonstration of time dissemination must fulfill criteria demonstrated in Table 1 in Polman et al. (2005).

<sup>f</sup> Abnormal VEP of the type seen in MS.

and Wingerchuk, 2006). Approximately 1 in 1,000 North Americans will develop MS over their lifetime. While women are 1.5 to 2 times as likely as men to develop MS, males with MS are likely to have a worse prognosis, more rapid disease progression, and later age of disease onset (Coyle, 2000). This evidence of gender dependent differences in disease outcomes suggests that there are sex-specific factors in the phenotypic variability and etiology of MS (Kantarci and Weinshenker, 2005). The etiology of MS is unknown, and the heterogeneous pathology of this disease suggests that several factors might be involved in the spectrum of idiopathic inflammatory demyelinating diseases (IDDs) that are encompassed in the diagnosis of MS (Hafler, 2004). It is believed that genetic, environmental, and immunological factors contribute collectively to the natural history and epidemiology of this disorder (Kurtzke and Wallin, 2000).

### 18.3.1. Genetic Epidemiology

Epidemiological, familial, and molecular studies of MS have supported a strong influence of genetic background on dis-

ease susceptibility (reviewed in Kurtzke and Wallin, 2000). The worldwide distribution of MS is skewed, with areas of high prevalence in North America, Europe, New Zealand, and Australia and areas of lower prevalence in South America, Asia, and Africa. In general, the prevalence and incidence of MS follow a north-south gradient in both hemispheres with individuals of Northern European ancestry more likely to be affected (Compston, 1994; Ebers and Sadovnick, 1994; Sadovnick et al., 1998). Therefore, the north-south gradient observed in the New World may reflect the propensity for individuals from regions of Europe with a high incidence of MS to migrate to the northern regions of the United States and Canada, and of individuals from regions of Europe with a lower incidence of MS to migrate to southern regions of the United States and South America (Ebers and Sadovnick, 1994; Sadovnick et al., 1998). In support of this theory, several studies have demonstrated different prevalences of MS among genetically disparate populations residing in the same geographic region. In a 1994 survey of MS in Australia, the prevalence of MS was found to be 37.1/100,000 in New South Wales and 29.4/100,000 in South Australia (McLeod et al.,

1994). Strikingly, no Aborigines or Torres Strait Islanders with MS were identified in this study (McLeod et al., 1994). In addition, the prevalence of MS is higher in Hungarians of Caucasian descent (37/100,000) than in Hungarian gypsies (2/100,000) (Kalman et al., 1991). Similarly, the prevalence of MS among people of Japanese decent living on the Pacific Coast (6.7/100,000) is considerably lower than that of Caucasians living in California (30/100,000). Of interest, it has been demonstrated that people of Japanese ancestry living on the West Coast of the United States have a slightly higher prevalence of MS than those living in Japan (2/100,000) suggesting that environmental factors also have a significant impact on disease susceptibility (Detels et al., 1977).

Family and twin studies clearly support a genetic influence in the development of MS. It has been demonstrated that biological relatives of MS patients have a greater likelihood of developing MS than adoptees and that conversely, family members of adopted individuals with MS do not have an increased risk of developing the disease (Ebers et al., 1995). Among biological relatives, the lifetime risk of developing MS increases with closer biological relationships and approximately 20% of MS patients have an affected family member (Sadovnick, 1993). First-degree relatives, particularly sisters, of an individual with MS are 20–40 times more likely to develop MS and the risk of MS decreases rapidly with second- and third-degree relatives (Sadovnick et al., 1998; Sadovnick and Ebers, 1993; Ebers et al., 1995). In addition, the rate of MS concordance is eight times greater in monozygotic than dizygotic twins. However, the concordance among monozygotic twins is only 25–30%, suggesting that genetic background is not sufficient to cause the disease (Bobowick et al., 1978). Therefore, a combined influence of genetic background and the environment in the development of MS has been proposed.

Considerable effort has been made to assign MS susceptibility to classical models of inheritance. However, the inheritance of MS does not conveniently fit any of these models. The ability to attribute a particular inheritance pattern to MS may be confounded by the difficulty in diagnosing this unpredictable disease. Moreover, because the age of risk ranges from the late teens to the late 50s, an individual cannot be considered unaffected with certitude until they are past the age of high risk. Over the years, several genes associated with immune function have been tentatively associated with an increased risk of MS. A strong association between MS and the major histocompatibility complex (MHC) class II alleles DRB1\*1501-DQB1\*0602 has been demonstrated (Lincoln et al., 2005). The MHC class I allele A201 has been shown to have protective effects (Sospedra and Martin, 2005). In addition, recent studies have suggested that polymorphisms of the cytokine interferon- $\gamma$  (IFN- $\gamma$ ) are associated with MS susceptibility in a gender dependent manner (Kantarci et al., 2005).

### 18.3.2. Environmental Epidemiology

The variation in disease incidence and prevalence according to geography (chiefly by distance from the equator), the influence of migration from low-to-high and high-to-low preva-

lence areas, and the observation of epidemics and clusters of MS all support an environmental influence on MS etiology (Kurtzke and Wallin, 2000). Migration studies based chiefly on Europeans who immigrated to South Africa, Israel, Australia, and Hawaii have also supported an infectious etiology of MS (Alter et al., 1978; Alter and Okihiro, 1971; Kurtzke et al., 1970; Casetta and Granieri, 2000). In general, individuals who migrate from high risk to low risk areas after the age of 15 maintain their risk of MS. However, individuals who migrate from high risk to low risk areas before the age of 15 acquire a lower risk. These data suggest that an environmental factor must be encountered before the age of 15 in order to influence MS susceptibility. In addition, examples of MS epidemics have been described in the Faroe Islands, the Shetland-Orkney Islands, Iceland, Sardinia, Key West Florida, Mossyrock Washington, South Africa, and Mansfield Massachusetts (Kurtzke, 1995; Pugliatti et al., 2002). Albeit rare, these epidemics support an infectious etiology of MS.

A wide range of environmental factors including vitamin D, smoking, exposure to solar ultraviolet radiation, exposure to organic solvents, household pets, and dietary fatty acids have been associated with the risk of developing MS (Soilu-Hanninen et al., 2005; Schiffer et al., 2001; Neuberger et al., 2004; Hernan et al., 2005; Riise et al., 2002). In addition, infectious agents have been suspected in the etiology of MS for over a century (Johnson, 1994). Dr. Pierre Marie, a pupil of Dr. Jean Martin Charcot who first classified MS and named the disorder *sclerose en plaque*, was the first to suggest an infectious etiology for MS. Marie hypothesized that MS was triggered by an infection that led to changes in blood vessels ultimately resulting in an inflammatory interstitial reaction of glial cells. In addition, Marie believed that many organisms were involved in the pathogenesis of MS based on the anecdotal association of acute infectious diseases (including malaria, typhoid, and childhood exanthem) with the onset of disease.

An infectious etiology of MS is consistent with a number of epidemiological observations as well as the pathological characteristics of this disease (reviewed in Soldan and Jacobson, 2004). It has been speculated that the infectious component in the development of MS might be a virus. Data implicating a virus in the pathogenesis of MS include: (a) geographic association of disease susceptibility with evidence of MS clustering (Haahr et al., 1997); (b) evidence that migration to and from high risk areas influences the likelihood of developing MS (Alter et al., 1978; Weinshenker, 1996); (c) abnormal immune responses to a variety of viruses; (d) epidemiological evidence of childhood exposure to infectious agents and an increase in disease exacerbations with viral infection (Johnson, 1994; Weinshenker, 1996); and (e) analogy with animal models and human diseases in which viruses can cause diseases with long incubation periods, a relapsing remitting course, and demyelination. It has been hypothesized that infectious diseases could induce MS either through molecular mimicry (the activation of autoreactive cells via cross-reactivity between foreign and



self-antigens), bystander activation (activation of autoreactive cells through nonspecific inflammation), or a combination of the two.

Viruses have been implicated in a number of demyelinating diseases of the CNS in humans and other animals. The association of viruses in other demyelinating diseases further suggests a viral influence in the development of MS, by demonstrating that viruses are capable of inducing demyelination, and that they can persist for years in the CNS presenting chronic diseases long after acute infection. Viruses involved in demyelinating diseases of humans and animals include JC virus, measles virus, HTLV-I, canine distemper virus (CDV), murine coronavirus (JHM strain), Theiler's mouse encephalomyelitis virus (TMEV), and Visna virus (reviewed in Soldan and Jacobson, 2004). Over the years, several viruses have been associated with MS, and these associations are based primarily on elevated antibody titers or the isolation of a particular virus from MS material. However, none of these viruses have demonstrated a definitive cause-effect relationship with the disease. Elevated antibody titers to several infectious agents including influenza C, herpes simplex (HSV), human herpes virus-6 (HHV-6), measles, varicella-zoster (VZV), rubeola, vaccinia, Epstein-Barr virus (EBV), simian virus 5 (SV5), multiple sclerosis retrovirus (MSRV), and *Chlamydia pneumoniae* have been reported (reviewed in Soldan and Jacobson, 2004). Although most of these reported agents have been discounted from consideration in the pathogenesis of MS, a few remain viable candidates.

### 18.3.3. Immunological Influences

In addition to genetic and environmental influences, it is widely accepted that T cell-mediated immune responses are involved in the etiology of MS (reviewed in Frohman et al., 2006a). This is based on the association of MS with genes involved with the immune response, the immunopathology of the disease, the clinical response of MS patients to immunosuppressive and immunomodulatory treatments, and similarities with experimental immune-mediated demyelinating diseases in animals.

A number of immune abnormalities are frequently observed in MS patients and lend support to an immunologic component of the MS disease process. One of the hallmarks against unknown CNS antigens of MS is the intrathecal secretion of oligoclonal antibodies, also known as oligoclonal bands (OCB) as seen by immunofixation electrophoretic separation. OCB are found in the CNS tissue and CSF of greater than 90% of MS patients (when determined by isoelectric focusing electrophoresis) (Fortini et al., 2003) and are helpful in confirming the diagnosis of the disease. OCB are not specific to MS as they are also found in several other chronic inflammatory CNS conditions of either infectious (CNS Lyme disease, bacterial meningitis, human immunodeficiency virus/HIV infection, neurosyphilis) or autoimmune (CNS lupus erythematosus, neurosarcoidosis) origin. Although OCB are not directed against a single antigen,

antibody bands specific for viral, bacterial, and self-antigens have been described (Sindic et al., 1994; Sriram et al., 1999). Therefore, it is unclear whether or not the intrathecal synthesis of immunoglobulins observed in MS results from the presence of disease-related lymphocytes or cells that are passively recruited into the CNS after the pathogenically relevant cells crossed the blood brain barrier (BBB).

In addition to the presence of OCB, other immunological markers of disease activity have been described in MS. The overexpression of several proinflammatory cytokines, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  (IFN- $\gamma$ ) have been demonstrated in MS (Ubogu et al., 2006; Frohman et al., 2006b). Treatment of MS patients with IFN- $\gamma$  resulted in a marked increase in exacerbations which supports the model of MS as an autoimmune disease mediated by T-helper-1 (TH-1) like T-cells. Furthermore, an increase in TNF- $\alpha$  expression has been found to precede relapses and inflammatory activity as measured by MRI, while the mRNA levels of inhibitory cytokines, such as interleukin-10 (IL-10) and transforming growth factor- $\beta$  (TGF- $\beta$ ), declined (Rieckmann et al., 1995). Recently expression of ADAM-17, a disintegrin and metalloproteinase that is the major proteinase responsible for the cleavage of membrane-bound TNF, was found to be upregulated in MS lesions (Plumb et al., 2006). The overexpression of these cytokines may be involved in disease pathogenesis by causing the upregulation of MHC and adhesion molecule expression on endothelial and glial cells, activation of macrophages and recruitment of TH-1 cells, or by damaging oligodendrocytes and myelin sheaths directly (Selmaj and Raine, 1988). In addition, peripheral blood mononuclear cells (PBMCs) from MS patients have been demonstrated to have increased numbers of chemokine receptor 5 (CCR5) producing cells (Strunk et al., 2000). However, individuals who fail to express a functional CCR5 receptor (due to a common deletion mutation, CCR5 delta 32, carried by 1% of the Caucasian population) are no more likely to develop MS than those with normal CCR5 expression (Bennetts et al., 1997). Finally, soluble adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and E-selectin are elevated in MS sera while soluble vascular cell adhesion molecules VCAM-1 and E-selectin are increased in the CSF of MS patients (Dore-Duffy et al., 1995).

Additional support for MS as a disease with an autoimmune component is provided by the clinical improvement obtained with immunomodulatory and anti-inflammatory therapies (Galetta et al., 2002). Although treatment with corticosteroids does not attenuate the long-term course of MS, it is used effectively in the treatment of MS exacerbations. It has been demonstrated that the administration of high-dose steroids immediately stops blood-brain barrier leakage as visualized by gadolinium-enhanced magnetic resonance imaging (MRI) (Simon, 2000). A number of immunosuppressive and chemotherapeutic drugs including cyclophosphamide, and methotrexate have been used in the treatment of MS with variable success. Other immunomodulatory therapies recently used in MS including interferon- $\beta$  (IFN- $\beta$ ), Copolymer-1 (COP-1)

and the humanized monoclonal antibody to the  $\alpha 4$ -integrin of the VLA-4 adhesion molecule (Tysabri [Antegren or Natalizumab]) are reviewed in Chap. 11.

## 18.4. Pathophysiology of MS

### 18.4.1. Histology and Physiology of MS Lesions

The hallmark of MS is the white matter “plaque,” an area of demyelination usually around a blood vessel with perivenular cell infiltration and gliosis (Frohman et al., 2006a). Recent studies have shown that axonal injury is common in chronic plaques and is an important determinant of permanent loss of neurologic function and disability (Trapp et al., 1999). The analysis of biopsy specimens collected for differential diagnosis and autopsy cases from patients with MS with at least one lesion in the active stages of demyelination has demonstrated four histologically-defined patterns of demyelination, Patterns I-IV (Lucchinetti et al., 2000). All four patterns demonstrate macrophage and T-cell inflammation, although they vary in associated pathological findings. Patterns I and II share macrophage and T-cell-associated inflammation and are described as follows: Pattern I (“macrophage mediated,” macrophage- and T-cell associated demyelination, perhaps dominated by products of activated macrophages), Pattern II (“antibody mediated,” same infiltrates as I, but with prominent IgG and complement/C9neo deposition). These patterns are largely restricted to small veins and venules and are suggestive of T-cell initiated processes in which macrophages and/or antibodies are important effector functions. They are felt to be immunopathologically closest to EAE. Lesions in Patterns III and IV are not typically seen in EAE. Pattern III (“distal oligodendroglipathy”) and Pattern IV (“primary oligodendrocyte damage with secondary demyelination”) also demonstrate inflammatory macrophages and T-cells, but deposition of IgG and complement are absent. Pattern III shows degeneration of distal oligodendrocyte processes, loss of oligodendrocytes by apoptosis at the plaque border, and loss of MAG (myelin associated glycoprotein) expression with preservation of PLP (proteolipid protein), MBP (myelin basic protein) and CNPase (cyclic nucleotide phosphodiesterase) staining. This pattern might represent a T-cell-mediated small vessel vasculitis with secondary ischemic damage to the white matter. However, demyelination in Pattern III is distinct from that in I and II in not being centered around veins and venules. Pattern IV also demonstrates prominent oligodendrocyte degeneration without apoptosis, and demyelination may be induced by T-cell and macrophages on the background of metabolically impaired oligodendrocytes. This pattern might be restricted to individuals with the primary progressive form of MS. Remarkably this study found no evidence for intra-individual variability in the plaque morphology: plaques within an individual patient showed similar morphology.

The net physiological consequences of immune-mediated myelin disruption are partial or total conduction block, slowing of conduction, and ephaptic cross-activation due to disruption of salutatory conduction mediated by the nodes of

Ranvier in myelinated fibers (reviewed in McDonald, 1998). Demyelinated fibers are incapable of transmitting impulse trains at physiological frequencies, and such a defect likely contributes to the sensory and motor disturbances seen in MS. Such conduction delays are often detected in the optic nerves through visual evoked potential (VEP) testing (McDonald, 1976). An interesting form of intermittent conduction block that is characteristic of multiple sclerosis is that precipitated by changes in temperature. Heat-induced visual acuity loss (Uhthoff’s phenomenon), weakness, and fatigue have all been attributed to heat-induced conduction disturbances, and such symptoms often resolve through decreasing body temperature (Guthrie and Nelson, 1995).

### 18.4.2. Immunopathogenesis of Myelin Damage in MS

Although the etiology of MS is unknown, there is considerable consensus on the role of immune-mediated mechanisms in disease pathogenesis. The following evidence has led to the concept that helper CD4+ T cells coordinate specific autoimmune responses to one or more candidate autoantigens of central myelin: (1) in the white matter lesions, perivenular infiltrates mainly contain lymphomononuclear cells, including CD4+ and CD8+ T cells, macrophages, and B cells; (2) the animal models of MS are primarily mediated by CD4+ T cells, although the humoral immune response also contributes to the pathogenesis; (3) effective treatments seem to preferentially target T-cell responses; and (4) disease susceptibility is influenced by genes that control presentation of antigens to T cells, such as those of the major histocompatibility complex (MHC, HLA in humans).

In addition to the role of CD4+ TH1 cells, recent studies have provided evidence for the involvement of CD8+ T cells and B cells in the pathogenesis of MS (Babbe et al., 2000; Goverman et al., 2005). Clonal expansion of CD8+ T cells, a subset of T cells with cytotoxic functions, has been recently shown to be predominant over CD4+ T cell expansions both in the brain and the spinal fluid of patients with MS (Babbe et al., 2000). Furthermore, the accumulation of B-cell clonotypes in CNS lesions and the spinal fluid of patients suggests that B cells may be chronically stimulated by CNS antigens leading to the synthesis of oligoclonal IgG (Goverman et al., 2005). Myelin-specific antibodies can contribute to myelin damage by inducing complement activation and facilitating macrophage-mediated myelin toxicity. Therefore, demyelination can be mediated through T-cells and macrophages as well as through complement activation. Myelin repair, which is limited *in vivo*, can be promoted *in vitro* with various growth factors (neurotrophin-3, platelet-derived growth factor, glial growth factor-2, others) (reviewed in Lubetzki et al., 2005).

A schematic view on the main processes that lead to autoimmune central demyelination is presented in Figures 18.2 and 18.3. Autoreactive, myelin antigen-specific T cells are part of the normal T-cell repertoire, although at low frequency. These cells are thought to be activated in the peripheral immune system

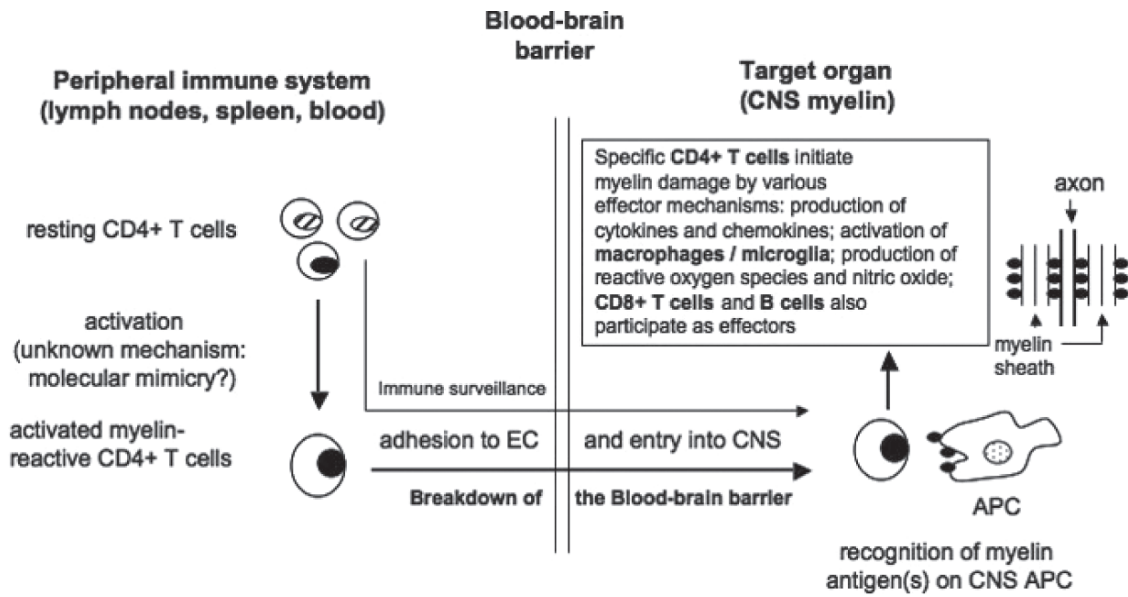


FIGURE 18.2. The immunopathogenesis of MS. A limited number of resting T cells may enter the CNS after crossing the intact blood-brain barrier (BBB). Autoreactive CD4+ T cells may be activated in the periphery by antigens that are structurally similar to myelin antigens (molecular mimicry). When activated T lymphocytes interact with endothelial cells (EC) of CNS venules, cell trafficking across the BBB increases. Reactivation of myelin antigen-specific T cells by local antigen-presenting cells (APC) may initiate myelin damage by several mechanisms: CD4+ T cell production of proinflammatory cytokines and chemokines that further recruit effector cells through an inflamed BBB; CD8+ T inducing cytolysis and chemokine production; activated macrophages producing oxygen radicals and NO (nitric oxide); deposition of myelin antigen-specific antibodies activating complement and facilitating myelin damage by activated macrophages. Resolution of inflammatory damage may be modulated by regulatory cells, that produce anti-inflammatory cytokines, and by elimination of effector cells by apoptosis.

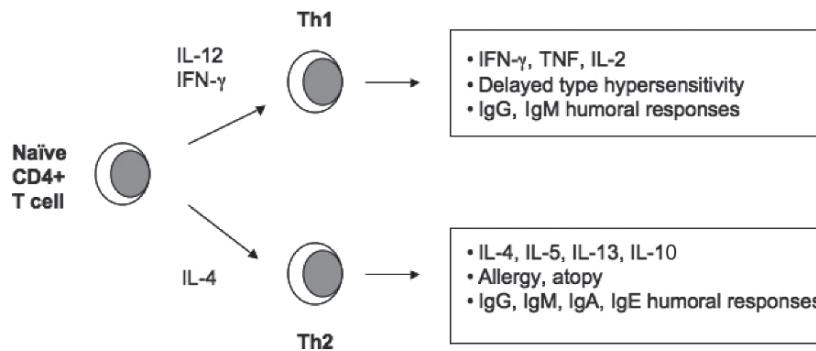


FIGURE 18.3. Comparison of Th1 and Th2 CD4+ T helper cell responses. Th1 and Th2 cells can be discriminated by their ability to produce different patterns of cytokines. Based mainly on the type, dose and route of administration of antigens, the genetic background of the host, and the cytokines present in the microenvironment, naive CD4+ T-cell precursors can develop into T-helper 1 (Th1) or Th2 cells. IL-12 (produced by macrophages) is the dominant factor promoting development of Th1 cells that are involved in cell-mediated immunity to intracellular pathogens and several instances of organ-specific autoimmunity. Th1 development also depends on IFN- $\gamma$  (mainly produced by T cells). In contrast, early exposure of naive CD4+ T-cell precursors to IL-4 results in the development of Th2 cells, which are involved in atopic and allergic reactions, and in certain types of systemic, antibody-mediated autoimmunity. Regulatory cells that produce the immunomodulatory cytokine TGF- $\beta$  can modulate the development of both Th1 and Th2 cells and the immunopathology they can induce.

(lymph nodes and spleen) of MS patients by unknown mechanisms. Structural similarity of foreign antigens (such as viral peptides) and myelin proteins may lead to their cross-recognition by

myelin-reactive T cells (molecular mimicry). Activated T cells can adhere to endothelial cells in cerebral blood vessels, cross the blood-brain barrier and enter the brain parenchyma. Proteolytic

enzymes such as matrix metalloproteases cleave extracellular matrix proteins and allow T-cell entry into the CNS. Upon reactivation by recognition of myelin antigens presented by microglia astrocytes, autoreactive T cells secrete proinflammatory cytokines that may cause direct damage to myelin, such as TNF- $\alpha$  and lymphotoxin, and chemokines that recruit effector cells, such as other T cells and monocytes-macrophages (Figure 18.3).

Recruitment of effector cells by chemokine-regulated chemotaxis of leukocytes and monocyte-macrophages is considered to be critical for effector cell entry into, and migration within, the CNS, each of which is a critical step in the pathogenesis of immune-mediated demyelination in multiple sclerosis (Ransohoff, 1999; Sorensen et al., 1999; Charo and Ransohoff, 2006; Ubogu et al., 2006). Chemokines represent a family of chemotactic cytokines that are comprised of two major subfamilies: the C-X-C ( $\alpha$  chemokine) subfamily that possesses two conserved N-terminal cysteine residues separated by a single amino acid, and the C-C ( $\beta$  chemokine) subfamily that possesses two adjacent cysteine residues. Chemokine-mediated adhesion of cells (tethering), extravasation, and infiltration is a complex process involving multiple signals and receptor-ligand interactions, and it is clear that several major steps are required: chemotaxis, adhesion and transmigration. In individuals with MS as well as in individuals without MS, most lymphocytes within the CSF are CD4+ memory T-cells in proportions that are higher than in the blood (Kivisakk et al., 2004). Such cells consistently express the chemokine receptor CCR7 and most also express CXCR3. Within perivascular MS lesions, however, CXCR3-expressing T-cells are commonly found while CCR7 expression is down-regulated (Sorensen et al., 1999).

Analysis of both CSF and brain tissue from individuals with MS has revealed consistent patterns of chemokine expression that distinguish not only MS patients from neurologically normal controls, but also stable MS patients from those undergoing acute relapses (Ransohoff, 1999; Sorensen et al., 1999). Levels of CXCL10 (CXCR3 ligand) and CCL5 (CCR5 ligand) were undetectable in CSF from more than 90% of control patients, but were elevated in more than 50% of CSF specimens from individuals with MS clinical attacks (Ransohoff, 1999). Furthermore, levels of CCL2 were significantly reduced during acute attacks, while levels of several other chemokines (CCL3, CXCL9, CXCL1, and CXCL8) were not significantly altered. This represented the first demonstration of CNS chemokine alterations related to MS clinical disease activity, and it suggested that TH-1-mediated T-cell signaling clearly plays a role in MS pathogenesis. As a result of such studies in MS patients, clinical trials with selected chemokine receptor antagonists have been undertaken (e.g. a CCR2 antagonist) (Charo and Ransohoff, 2006).

Another critical determinant in the cascade of events leading to MS lesion development is antigen presentation. Antigen presenting cell (APC)/T-cell activation involves at least four major processes: (1) Molecular mimicry, (2) Epitope spreading, (3) Bystander activation, and (4) Cryptic antigen

presentation. These mechanisms are not mutually exclusive and each is believed to contribute to the pathogenesis of MS. Molecular mimicry, as described earlier in this chapter, is a phenomenon wherein foreign antigens have enough similarity to the host's endogenous proteins to elicit an autoimmune response. In 1985, it was demonstrated that injection of rabbits with a Hepatitis B virus peptide containing six amino acids in common with MBP induces encephalitis through a cross-reactive immune reaction against MBP (Fujinami and Oldstone, 1985). Since this early observation, the concept of molecular mimicry has been expanded to include involvement of sequence homology, sharing of conserved TCR (T cell receptor) and MHC contact motifs between peptides, additive stimulatory contributions, and structural homology (reviewed in Sospedra and Martin, 2006). Of interest, regions of sequence and structural homology between MBP and two human herpesviruses that are candidate etiologic agents for MS, HHV-6 and EBV have recently been identified (Tejada-Simon et al., 2003; Holmoy et al., 2004).

Epitope spreading occurs when the immune response to a target antigen extends to other epitopes present on the cell that are involved in the primary immune response. These additional epitopes may be within the same molecule (intramolecular) or in different molecules (intermolecular) (Vanderlugt and Miller, 2002). T-cell activation and functional epitope spreading require costimulation via CD28-CD80/86 or CD154-CD40 interactions (Vanderlugt and Miller, 2002). The pathological consequences of epitope spreading have been demonstrated in several mouse models for MS, including Experimental Autoimmune Encephalitis (EAE) and Theiler's Murine Encephalitis Virus (TMEV) induced demyelinating disease (discussed later in this chapter). In EAE, priming with the PLP immunodominant epitope results in an acute clinical episode that is followed by a relapsing remitting disease course that is mediated by PLP specific TH-1 cells and correlates with intramolecular epitope spreading (Vanderlugt and Miller, 2002). In Theiler's virus-induced demyelinating disease, disease onset occurs after infection with TMEV, resulting in a chronic disease course. As the disease progresses, TH-1 responses to TMEV persist. In addition, myelin debris released as a result of the initial tissue damage induces CD4+ TH-1 responses to myelin epitopes starting with PLP and, in late stages of the disease, spreading to MBP and MOG (myelin oligodendrocyte glycoprotein) (Vanderlugt and Miller, 2002).

Bystander activation activation of autoreactive cells through nonspecific inflammation and induction of inflammatory cytokines and chemokines is also of pathological consequence in MS. It has been suggested that bystander activation, induced by persistent virus infection or primed by molecular mimicry may activate autoreactive T-cells specific for the CNS (McCoy et al., 2006). Finally, cryptic antigens may also play a role in immune activation. In other immune-mediated diseases such as Chronic Lymphocytic Thyroiditis and Chagas Heart Disease, exposure of cryptic epitopes leads to the activation of autoimmune cells and further contributes to



the pathogenesis (Rose and Burek, 2000; Leon and Engman, 2003). Similarly, it has been suggested that myelin destruction leads to the exposure of cryptic axonal antigens that are normally shielded from immune surveillance by the tightly sealed paranodal loops of myelin. The exposure of these cryptic antigens may activate auto-reactive T-cells and the production of anti-axolemmal antibodies. It has been suggested that these anti-axolemmal antibodies induce neurodegeneration, prevent remyelination and contribute to the axonal loss that correlates with functional loss in MS. (DeVries, 2004).

## 18.5. Animal Models of MS

### 18.5.1. Experimental Autoimmune Encephalomyelitis (EAE)

While no single animal model completely recapitulates all features of MS, Experimental autoimmune encephalomyelitis (EAE, Table 18.2) is the most widely used. Rivers' seminal investigation into the iatrogenic human disease, postvaccinal encephalomyelitis was critical in the development of field of EAE (Rivers et al., 1933). Early models of EAE involved the induction of inflammatory demyelinating lesions in experimental animals by immunization with brain or spinal cord tissue (Rivers et al., 1933). Current models rely on active immunization with myelin antigens (myelin basic protein (MBP), myelin associated glycoprotein (MAG), proteolipid protein (PLP) or myelin-oligodendrocyte-glycoprotein (MOG)) in adjuvant or by adoptive transfer of encephalitogenic, myelin antigen specific T cell lines or clones (passive immunization) (Ebers, 1999; Lassmann and Wekerle, 1998; Ercolini and Miller, 2006). Although mouse and rat models are the most commonly used, EAE can be induced in a variety of susceptible animals including guinea pigs, rabbits, pigs, and monkeys.

The clinical signs of EAE reflect the acute inflammatory responses developing in the brain and spinal cord: hindlimb and tail paralysis, quadriparesis, ataxia, abnormal righting responses, and sometimes incontinence (Lublin, 1996). These episodes, whether associated with active or passive immunization, are associated with infiltration of myelin-specific inflammatory TH1 CD4+ T-cells into the CNS (McRae et al., 1992). The disease can be either monophasic (with a single paralytic episode followed by recovery), relapsing-remitting (with repeated cycles of paralysis interrupted with partial or full recovery), or chronic (initial symptoms either stabilize or progress) (Ercolini and Miller, 2006). These later two clinical courses more closely resemble the natural history of MS symptoms. In EAE, TH-1, proinflammatory cytokines contribute to tissue destruction directly, upregulate expression of MHC I and II within the CNS, and lead to macrophage and microglial activation. Paradoxically, treatment with the proinflammatory cytokines IFN- $\gamma$  and TNF- $\alpha$  is protective in EAE, while similar treatments lead to disease exacerbation in MS (Krakowski and Owens, 1996).

EAE is traditionally regarded as a prototypic TH-1 CD4+ T-cell mediated autoimmune disease of the CNS. However, other cells of the immune system play important roles in the neuropathogenesis of this disorder. Defining the precise role of various cell types in EAE is complicated by the diverse and heterogeneous nature of EAE model systems. Recently, a new class of T-cells, TH-17 cells, has been shown to regulate inflammation in EAE. This unique subset of T helper cells produce IL-17 and develops along a pathway that is distinct from that of TH-1 and TH-2 cell differentiation (Park et al., 2005). It has been demonstrated that neutralization of IL-17 with IL-17-receptor-Fc-protein or an IL-17 monoclonal antibody ameliorates the disease course of EAE (Hofstetter et al., 2005). The production of IL-17 requires upstream activation of IL-23, and neutralizing antibodies specific for

TABLE 18.2. Features of EAE and MS.

Feature	EAE	MS
CNS signs	+++	+++
Relapsing disease	++	+++
Perivascular inflammation	+++	+++
Cellular infiltrate	CD4+ T-cells, MOG-specific CD8+ T-cells	CD8+ T-cells, CD4+ T-cells, B-cells
Demyelination	+, +++, perivenous	+++; diffuse
Remyelination	++	++
Immunogen	MBP, PLP, MOG, others	Unknown
CSF immunology	Antibodies to myelin antigens	Rarely find antibodies to myelin antigens; OCB antigens unknown
Genetic predisposition	++	++
Linked to MHC	++	++
Response to immunomodulation	+++	++

Source: Adapted from Lublin (1996) and Sriram and Steiner (2005).

IL-23 also ameliorate disease progression in EAE (Chen et al., 2006). In addition, EAE is significantly suppressed in IL-17 knockout mice further indicating the importance of IL-17 in the pathogenesis of EAE (Komiyama et al., 2006).

In addition to T-cells, other cell types have been found to play important roles in EAE induced by both active immunization and adoptive transfer. CD8+ cells play a variable role in EAE. In the absence of CD8+ T-cells, more severe disease is observed. However, MOG-specific CD8+ T-cells have been observed and are capable of transferring EAE in SCID mice. In addition, H2k MBP-specific CD8+ T-cell clones recognizing the peptide fragment MBP 79–87 (Huseby et al., 1999). B cells are generally believed to be dispensable in EAE as B-cell deficient (B10.PL × SJL/J) mice develop EAE after immunization (Dittel et al., 2000). In B6 mice, the absence of B-cells exacerbates EAE suggesting a protective role for these cells. However, MOG-induced EAE is aggravated by administration of anti-MOG antibodies, suggesting an important role for the humoral response in some models of EAE.

EAE has been used extensively to gain insight into almost every aspect of the neuroimmunology and neuropathology of MS. In addition, the majority of therapies that are either routinely used or currently under development for MS were initially developed in EAE. However, EAE has received criticism as an incomplete model of MS (Sriram and Steiner, 2005). It has been suggested that EAE is a model of acute CNS inflammation/demyelination such as acute disseminated encephalomyelitis (ADEM) rather than a true counterpart for MS. Critical differences between MS and EAE include differences in lesion composition (CD4+ cells dominate in EAE while in the inflammatory MS lesion macrophages and CD8+ cells are frequently observed), lesion location, affect of immunotherapies (e.g. IFN- $\gamma$  can ameliorate EAE, but increases exacerbations in MS, IFN- $\beta$  decreases relapse rate in MS, but can worsen EAE), and the observation that the significant axonal damage observed in MS is absent in most models of EAE (Sriram and Steiner, 2005). Nevertheless, the three dominant, FDA approved treatment modalities for MS (Copolymer-1, IFN- $\beta$ , and Natalizumab) were all developed in EAE which underscores the great utility of this model in spite of its imperfections (Steinman and Zamvil, 2006).

## 18.5.2. Viral Models of MS

### 18.5.2.1. Theiler's Murine Encephalomyelitis Virus

There are two major virus induced, murine models of MS: Theiler's Murine encephalomyelitis viruses (TMEV) and mouse hepatitis virus (MHV). Each is a naturally occurring rodent virus that infects the CNS and induces demyelination in genetically susceptible hosts. Theiler's Murine encephalomyelitis viruses (TMEV) are members of the *Picornaviridae* that naturally cause neurologic and enteric diseases in mice (Rodriguez et al., 1987). Several strains of TMEV are capable of inducing paralysis in susceptible rodents. Interestingly it is the persistent, avirulent strain that causes chronic disease;

members of the GDVII subgroup grow to high titers and cause a fatal polioencephalomyelitis, while the Daniels (DA) strain and other members of the Theiler's original (TO) subgroup grow to relatively low titers and induce a chronic inflammatory demyelinating disease of the spinal cord (Rodriguez and Roos, 1992). In the later case, the disease is bi-phasic with an acute encephalitic phase followed by late chronic demyelinating disease, associated with mononuclear infiltrates and demyelinating lesions (Murray et al., 2000).

Several features of TMEV-induced CNS disease including clinical, genetic, and histopathological similarities with MS make this a relevant experimental model. Chronic and/or relapsing remitting paralytic symptoms are observed in both MS and TMEV encephalomyelitis. In addition, natural infection with the TO strains of TMEV progresses to CNS infection and the development of chronic, progressive neurologic disease in a small percentage of infected mice and intracerebral injection only leads to CNS disease in some mouse strains, suggesting a genetic component to the disease. Both diseases are under multigenic control and susceptibility is associated with genes involved in the immune response including MHC genes (Borrow et al., 1992). In addition, TMEV encephalomyelitis, like MS, is a predominantly TH-1 mediated disorder. A strong, TH-1 response has been demonstrated during the acute phase (Chang et al., 2000) and, in some mouse strains, during the chronic phase (Chang et al., 2000). Recently, matrix metalloproteinases, which degrade extracellular matrix molecules and are involved in demyelination processes, were demonstrated to be upregulated in chronic demyelinating Theiler's Murine encephalomyelitis (Ulrich et al., 2006). Lastly, the cellular makeup of the mononuclear infiltrates in both diseases consists primarily of T lymphocytes, monocytes, and macrophages (the primary host cell of the virus) and demonstrate striking similarities with MS lesions. It has been demonstrated that CD8+ T cells are critical in the prevention of TMEV mediated demyelination and that CD4+ cells are important in the synthesis of neutralizing antibodies. In addition, as TMEV encephalomyelitis is caused by latent and persistent infection of the CNS, it makes this an intriguing model for MS, a disorder for which an infectious etiology is suspected. Recently, recombinant TMEV encoding a mimic peptide for PLP that is naturally expressed by *Haemophilus influenzae* has been described (Olson et al., 2005). Infection with this recombinant virus results in early disease onset. These studies support molecular mimicry as a viable hypothesis in MS.

### 18.5.2.2. Murine Hepatitis Virus

Murine hepatitis virus (MHV) is a murine coronavirus that infects the liver. Two strains of MHV, JHM and MHC-A59 are neurotropic variants that are frequently used in studies of MHV infection of the CNS (Matthews et al., 2002). Both viruses readily infect oligodendrocytes, astrocytes and neurons (Matthews et al., 2002). After acute infection, the animals develop a chronic progressive neurologic disease characterized by a single major episode of demyelination

and accompanied by hind limb paresis, paralysis, and ataxia. Recovery is mediated by CNS and, sometimes, peripheral nerve remyelination (Matthews et al., 2002). In this model, it is believed that demyelination results from both immune-mediated and direct viral destruction. CD8+ T-cells mediate CNS disease and MHV induces both TH-1 and TH-2 cytokines. Interestingly, partial depletion of CD4+ and CD8+ cells does not eliminate chronic demyelination. In addition, humoral immunity is critical for both clearance of the virus and establishment of persistent infection. Chronic lesions in MHV are found throughout the spinal cord and are similar to chronic lesions in MS. As the disease progresses, lymphocytic infiltration diminishes while demyelination and astrogliosis increases. Unlike TMEV, no autoimmune reaction against brain antigens has been described in the MHV model.

## 18.6. Recent Advances in the Neuroimmunology of MS

### 18.6.1. CD4+CD25+ Regulatory T-Cells (T Regs)

While CD4+ T cells are traditionally regarded as pathogenic in MS, CD4+CD25+ regulatory T-cells (T regs) have emerged as major players in inhibiting autoimmune disease in humans and in rodent models. Suppression by T regs is critical in preventing autoreactive T-cells from causing autoimmune disorders and in inducing peripheral tolerance. Accordingly, depletion of T reg cells leads to the onset of systemic autoimmune disorders in mice. Although T reg cells exist in the same frequency in MS patients, the effector function of these cells are significantly impaired in relapsing-remitting MS patients (Viglietta et al., 2004). Interestingly, T reg cell function and expression of the T reg-specific transcription factor, FOXP3, is normal in chronic progressive MS patients (Venken et al., 2006). In EAE, suboptimal T-cell stimulation results in significant pathology and delayed recovery upon depletion of CD4+CD25+FOXP3+ cells, indicating that these cells are highly important in raising the threshold for triggering autoreactive T-cell responses (Stephens et al., 2005). In the future, monitoring the effects of immunomodulatory therapies on T reg cells will help define the role of these cells in autoimmune disorders and lead to the development of novel therapies for MS. Currently, a novel T-cell receptor peptide-based immunotherapy (NeuroVax) that restores normal function of FOXP3+ T regs is entering phase II clinical trials (Darlington, 2005).

### 18.6.2. B Cell Responses in MS

While the presence of OCB in MS has long been appreciated, recent studies have re-examined the role of B-cells in MS. Antibody binding studies have demonstrated that binding to a neuronal cell line was increased in chronic progressive

MS patients compared to relapsing remitting multiple sclerosis patients and other inflammatory CNS disease controls (Lily et al., 2004). This approach could lead to the identification of cell surface autoantigens that may be involved in mediating demyelination or neuronal damage. In another study, proteomics technology was used to characterize autoantibodies directed against candidate antigens, in EAE, MS patients, and controls (Robinson et al., 2003).

In addition, antibodies may play a beneficial role in MS by skewing the immune system away from a TH-1 biased response and toward a TH-2 cytokine pattern or by fostering myelin repair. It has been demonstrated that IgM antibodies to CNS antigens enhance remyelination in MS (Sospedra and Martin, 2005). The beneficial effect of pooled intravenous Ig in MS therapy supports a beneficial role for antibodies in MS. It is believed that this beneficial effect occurs through several mechanisms including inactivation of cytokines, Fc-receptor blockade, blocking of CD4 and MHC, and modulation of apoptosis (Sospedra and Martin, 2005).

## 18.7. Other Demyelinating Diseases of the CNS

Several other demyelinating disorders such as neuromyelitis optica (NMO/Devic's disease), transverse myelitis, acute necrotizing myelitis and Foix-Alajuanine Syndrome share some features with multiple sclerosis but in some cases represent distinct pathological entities. Neuromyelitis optica that is characterized by a generally acute, severe clinical necrotizing demyelinating syndrome that primarily affects the optic nerves and spinal cord. Unlike classical MS-associated demyelination, the neuromyelitis optica lesions are characterized by necrosis and vascular proliferation associated with a marked elevation of the CSF white blood cell count ( $>100$  wbc/mm<sup>3</sup>; often neutrophils) and protein level. OCBs are usually not found in the CSF. Neuromyelitis optica is more often monophasic (35%) than is MS. Pathologically, it is characterized by gray and white matter necrosis, infiltration of macrophages, eosinophils and neutrophils, and deposition of IgM and IgG with perivascular complement activation (Kerr and Calabresi, 2005). Recent discovery of an association between the presence in the serum of an IgG antibody against aquaporin 4, a mercurial-insensitive water channel protein concentrated in astrocytic foot processes at the blood-brain barrier, in patients with neuromyelitis optica, has led to the investigation of an anti-B cell (anti-CD20) humanized monoclonal antibody as treatment for this disease (Pittock et al., 2006; Cree et al., 2005; Lennon et al., 2005).

Demyelinating syndromes such as optic neuritis and transverse myelitis have clearly defined relationships with MS and are felt to represent the typical demyelinating lesions found in the white matter of the brain in MS. Optic neuritis is frequently the initial clinical manifestation of MS, and is typically heralded by a decline in vision associated with eye pain over

a 7- to 10-day period (Frohman et al., 2005). Approximately 50–70% of individuals with optic neuritis have periventricular white matter abnormalities consistent with demyelination by MRI assessment, and up to 88% of these individuals develop definite MS within 14 years of presentation of optic neuritis (Brex et al., 2002). Patients respond well to acute corticosteroid treatment as well as prophylactic treatment with IFN- $\beta$ , similar to individuals with MS (Balcer, 2006). Transverse myelitis refers to the clinical syndrome of partial or complete spinal cord dysfunction resulting from inflammatory demyelination. With partial cord dysfunction, the risk of development of MS is as high as 70%, but it is less than 15% when spinal cord function is completely lost (Coyle, 2000). Transverse myelitis is associated with marked CSF elevations of IL-6, which in model systems can activate inducible nitric oxide synthase (iNOS) in the spinal cord, possibly leading to neuronal injury (Kerr and Calabresi, 2005).

The Foix-Alajuanine Syndrome and acute necrotizing myelitis are pathologically and clinically distinct from demyelinating syndromes associated with MS. Foix-Alajuanine Syndrome is a rare cause of myelopathy (spinal cord dysfunction) that is caused by a dural arteriovenous malformation of the spinal cord (Mishra and Kaw, 2005). It is often confused with transverse myelitis or acute necrotizing myelitis. Rather than immune-mediated cord damage, the syndrome is characterized by subarachnoid hemorrhages that result in severe local arachnoid fibrosis and associated thrombosis of local blood vessels. Patients are usually over 50 years of age, and suffer a slow progression to paraplegia. Acute necrotizing myelitis is distinct from typical demyelinating spinal cord involvement in MS. It is associated with coagulative necrosis of both gray and white matter, infiltration of T-cells and macrophages, a high CSF protein level (>500 mg/dl), and absence of OCB in the CSF, thus distinguishing it pathologically from MS, but suggesting important similarities with neuromyelitis optica (Katz and Ropper, 2000). Clearly, better understanding of the various forms of immune-mediated myelitis (neuromyelitis optica, transverse myelitis, necrotizing myelitis) will likely reveal both common and unique features of each that will guide the development of newer therapies for MS and other demyelinating diseases.

## Summary

The pathogenesis of MS is a complex immune-mediated process that can likely be triggered by multiple antigens presented within the CNS, which can elicit T-cell and B-cell responses that promote myelin damage and the resulting neuronal damage. Antigenic responses are thought to be induced and amplified in some cases through molecular mimicry, epitope spreading, bystander activation of antigen-presenting cells, and by cryptic antigens that are normally shielded from presentation. Epidemiological evidence suggests that environmental (infectious) agents can trigger such responses in genetically

susceptible hosts, based upon strong associations between geographic residence, occurrence of MS and presence of certain MHC I and II alleles within the affected population. Animal models of induced CNS demyelination (EAE, neurotropic viruses; TMEV, MHV) have largely confirmed that presentation of myelin-associated antigens within the CNS as well as CNS virus infection can elicit strong MS-like T-cell and B-cell responses and MS-like neurological dysfunction in animal hosts. Within the brains of MS patients, pathological studies confirm the infiltration of CD4+ and CD8+ T-cells and macrophages, as well as deposition of IgG and complement to varying degrees in defined subtypes of MS plaques. Along with such infiltrates, robust expression of proinflammatory cytokines and chemokines within the brain and CSF is consistently demonstrated, and such factors can promote the recruitment of reactive T cells and monocytes from the periphery that can amplify inflammatory responses. Clinical responses to different immunomodulating therapies in MS patients further support roles for T-cell- and B-cell-mediated immune dysregulation in MS pathogenesis. Future MS therapies will likely target not only activated lymphocytes (T-cells, B-cells, Tregs) and macrophages, but also the neurons that are rendered vulnerable by associated myelin damage.

## Review Questions/Problems

- Which of the following statements is true about gender differences in MS?**
  - Men are more likely than women to develop MS.
  - Women are likely to have a later age of disease onset.
  - Women have a more rapid disease progression
  - Men with MS have a worse prognosis.
- The geographic distribution of MS:**
  - follows an east-west gradient
  - increases at higher altitudes
  - increases with distance from the equator
  - is skewed toward northern latitudes in both hemispheres
- An environmental component in the etiology of MS is supported by:**
  - the geographic distribution of MS
  - migration studies
  - relatively low concordance (25–30%) in identical twins
  - reports of MS epidemics
  - all of the above
- Which of the following immune abnormalities are not associated with MS?**
  - overexpression of IL-10
  - increased expression of adhesion molecules
  - presence of oligoclonal bands in the CSF
  - increased TNF- $\alpha$  expression preceding clinical relapse



- 5. Which immunomodulatory therapies are frequently used in MS?**
- IFN- $\beta$
  - IFN- $\gamma$
  - Copolymer-1
  - corticosteroids
  - all of the above
  - a, b & d
- 6. Oligoclonal bands represent:**
- Immunoglobulins directed against recently identified myelin epitopes in MS
  - Immunoglobulins directed against unknown CNS epitopes in MS
  - Immunoglobulins that have been shown to deposit around demyelinated plaques in MS
  - Immunoglobulins often detected in the serum of individuals with MS
  - b & e
- 7. MS plaques have been histologically demonstrated to include:**
- infiltration of CD8+ T-cells
  - infiltration of CD4+ T-cells
  - infiltration of B-cells
  - IgG deposition
  - Complement deposition
  - all of the above
  - a & b
  - a, b & c
  - a, b, c & d
- 8. Which of the following statements is false?**
- In MS plaques perivenular infiltrates contain mainly mononuclear and lymphocytic cells, including CD8+ T-cells, CD4+ T-cells, macrophages, and B-cells.
  - In the EAE model, the primary infiltrating cell is the CD8+ T-cell.
  - Effective MS treatments generally target T-cell responses
  - In both EAE and MS, disease susceptibility is influenced by genes that control presentation of antigens to T-cells.
- 9. In the pathogenesis of MS, molecular mimicry implies:**
- Receptors mediating T-cell migration have overlapping function, i.e., they can mimic each other's ligand specificity.
  - Immune modulating chemokines or cytokines can mimic the molecular functions of each other.
  - Structural similarity of foreign antigens and myelin protein components may lead to cross-recognition by myelin-reactive T-cells.
  - Suppression of selected T-cell responses can have a global impact on both CD4+ and CD8+ T-cells.
- 10. Comparison of EAE with MS shows the following:**
- Both demonstrate monophasic, relapsing, and chronic progressive clinical courses
  - Perivascular inflammation is common in both.
  - Demyelination is diffuse throughout the brain in both.
  - All of the above
  - a & b
- 11. Which viruses have been associated with an increased risk for development of MS?**
- Herpes simplex (HSV)
  - Human herpes virus-6 (HHV-6)
  - Measles
  - Varicella-zoster
  - Rubeola
  - Epstein-Barr
  - All of the above
  - a, b, c & f
- 12. Among the viruses listed in question 11, which ones have been demonstrated to be causative agents in MS?**
- a
  - b
  - c
  - d
  - e
  - f
  - all of the above
  - none of the above
- 13. Which features of Theiler's virus-induced CNS disease make it a useful model for MS?**
- The animals develop chronic and/or relapsing paralytic symptoms.
  - Both diseases are under multigenic control by immune response genes.
  - Both are predominantly TH-1 mediated disorders.
  - B-cell infiltration predominates in Theiler's virus induced disease.
  - a
  - a & b
  - a, b & c
  - a, b, c & d
- 14. Apoptosis of which cell type is a histological hallmark of at least one pathologic subtype of MS plaque?**
- neuron
  - astrocyte
  - oligodendrocyte
  - macrophage
  - none of the above
- 15. Optic neuritis is the presenting symptom in what percentage of MS patients?**
- 10%

- b. 20%
- c. 30%
- d. 40%
- e. 50%

#### 16. The diagnostic criteria for MS:

- a. Depend upon the demonstration of white matter abnormalities in the brain or spinal cord by MRI.
- b. Require evidence for the presence of neurological dysfunction for the diagnosis in all cases.
- c. Require that there is no better explanation (other than MS) for the clinical presentation.
- d. None of the above
- e. a, b & c
- f. a & c
- g. b & c

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