

BIOLOGY OF DISEASE

Multiple Sclerosis: Current Pathophysiological Concepts

Dean M. Wingerchuk, Claudia F. Lucchinetti, and John H. Noseworthy

Department of Neurology (DMW), Mayo Clinic, Scottsdale, Arizona, and Department of Neurology (CFL, JHN), Mayo Clinic, Rochester, Minnesota

SUMMARY: Multiple sclerosis (MS) is an often disabling disease primarily affecting young adults that exhibits extraordinary clinical, radiological, and pathological heterogeneity. We review the following: (a) known environmental and genetic factors that contribute to MS susceptibility; (b) current knowledge regarding fundamental pathophysiological processes in MS, including immune cell recruitment and entry into the central nervous system (CNS), formation of the plaque, and orchestration of the immune response; (c) descriptive and qualitative distinct pathological patterns in MS and their implications; (d) the evidence supporting the causative role of direct toxins, cell-mediated and humorally mediated immune mechanisms, and the concept of a "primary oligodendroglialopathy" in demyelination and axonal injury; (e) the potential benefits of inflammation; (f) the prospects for remyelination; and (g) therapeutic implications and approaches suggested by putative pathophysiological mechanisms. (*Lab Invest* 2001, 81:263–281).

Multiple sclerosis (MS) is a common, heterogeneous disorder of the central nervous system (CNS) (Noseworthy, 1999; Noseworthy et al, 2000a). Its causes and the factors that contribute to its heterogeneity are largely unknown, although it is likely a complex trait with genetic and environmental components. The disease affects about 0.1% of the population in temperate climates, some 250,000 to 350,000 people in the United States. It is a disease of young people (median age of onset is approximately 28 years) but is lifelong and is often disabling; 50% of patients require a cane to walk 15 years after disease onset (Weinshenker et al, 1989).

Early in the course of relapsing-remitting disease (RRMS), which affects about 85% of patients, neurological symptoms and signs develop over several days, plateau, and then usually improve over days to weeks (Schumacher et al, 1965). These relapses typically consist of one or a combination of the following: sensory symptoms, optic neuritis, Lhermitte's sign (axial or limb paresthesias with neck flexion), limb weakness, gait ataxia, brain stem symptoms (diplopia; ataxia), Uhthoff symptom (symptomatic worsening with increases in body temperature), a circadian fatigue pattern (fatigue worse in mid- to late-afternoon concomitant with increases in core body temperature), and sphincter dysfunction. Inflammatory infiltrates and

demyelination in brain and spinal cord white matter usually accompany these clinical exacerbations. Periods of clinical quiescence (remissions) occur between exacerbations; remissions vary in length and may last several years but are infrequently permanent. The remaining 15% of patients begin the disease course by experiencing gradually progressive neurological function, typically a slowly worsening myelopathy (primary progressive disease, PPMS). Approximately two-thirds of patients with RRMS eventually undergo a similar fate; as relapse frequency lessens over time, progressive neurological dysfunction emerges, signaling the development of secondary progressive disease (SPMS) (Weinshenker et al, 1989). Some patients who convert to a secondary progressive course continue to experience superimposed relapses.

The above classification system defines the prototypic or classic form of MS (Lublin and Reingold, 1996). Classification schemes for CNS demyelinating diseases include several uncommon syndromes with controversial relationships to classic MS, including complete transverse myelitis, neuromyelitis optica (Devic's syndrome), acute disseminated encephalomyelitis, Balo's concentric sclerosis, and the fulminant Marburg variant (Korte et al, 1994; Mendez and Pogacar, 1988; Wingerchuk et al, 1999). These syndromes retain the basic inflammatory and demyelinating pathology of MS but differ from classic disease with unusually acute and severe clinical presentations, restricted lesion topography (eg, optic nerve and spinal cord lesions in Devic's syndrome), or distinct pathological features (eg, pronounced acute axonal destruction and necrosis in neuromyelitis optica and

Received November 27, 2000.

Address reprint requests to: Dr. John H. Noseworthy, MD, FRCP(C), Mayo Clinic, 200 First Street SW, Rochester, MN 55905. E-mail: noseworthy.john@mayo.edu

the Balo's and Marburg variants). Apart from these entities, it has generally been accepted that similar pathophysiological mechanisms are operative in all patients with prototypic MS. Recent studies, however, suggest that pathological heterogeneity may also exist amongst patients with otherwise classic disease (Lucchinetti et al, 1996, 2000a). There may be a restricted number of distinct pathological patterns with a single, dominant pattern present in all active lesions within an individual patient. This finding suggests that distinct pathogenetic mechanisms may be involved in different patient subgroups and has wide-ranging implications for disease classification and future investigation of the causes and pathophysiological mechanisms that underlie MS (Lucchinetti et al, 2000a). Furthermore, increasing attention is being paid to the role of axonal injury and loss, the likely correlate of progressive and irreparable injury in MS. We will review progress in the understanding of the etiology, pathophysiology, and pathology of MS and their implications for discovering effective treatments that arrest or repair damage done by this disabling disease.

Environment and Genetics

The cause of MS is not known. Epidemiological findings support both environmental and genetic hypotheses, and these forces likely interact to produce individual disease susceptibility and influence disease course.

Several observations seemingly support environmental hypotheses. The prevalence of MS generally increases with distance from the equator (Kurtzke, 1980), and apparent epidemics and clusters of MS have been reported. Migration (and age at migration) may modify the disease risk, and concordance rates in monozygotic twins do not exceed approximately 30% (Ebers et al, 1986; Mumford et al, 1994). Some consider these findings as supportive of an ecological or infectious hypothesis for MS susceptibility. It is unclear whether putative environmental factors are operative at the individual level (eg, infectious, transmissible agents) or elevate the risk of the entire population (eg, ecological factors, such as climate, soil conditions, or diet) (Lauer, 1997). Ecological case-control studies are often limited because exposures are usually similar amongst cases and controls. Isolation of infectious agents and/or serological evidence of greater exposure in MS cases compared to controls have been reported frequently over several decades. Recent reports implicate human herpes virus 6 (HHV-6) (Challoner et al, 1995; Friedman et al, 1999) and *Chlamydia pneumoniae* (Gilden, 1999; Sriram et al, 1999) as causative agents, but others have failed to confirm these observations (Boman et al 2000; Martin et al, 1997; Mirandola et al, 1999). To date, no single infectious agent has withstood the test of time.

Genetic predisposition to MS has been established from the following evidence: familial aggregation unexplained by environmental factors (Ebers et al, 1995); much higher monozygotic than dizygotic twin concordance rate (31% versus 5%) (Sadovnick et al, 1993);

ethnic predisposition (eg, Northern Europeans) and protection (many groups, including North American Indians and Hutterites, despite living in regions with high MS prevalence); and association with human leukocyte antigen (HLA) DR2. The exact mode of inheritance is unknown but does not appear to be Mendelian or mitochondrial in nature. In general, the risk to a first-degree relative is approximately 1% to 4% (10–40 times the population risk), but this value may be substantially higher in pedigrees with multiple affected members.

The genes that contribute to MS susceptibility have not been identified. The HLA DR2 allele has been associated with MS in many populations (Ebers et al, 1995). Four entire human genome screens by linkage have been reported (Ebers et al, 1996; Haines et al, 1996; Kuokkanen et al, 1997; Sawcer et al, 1996). Although refinement of the original genome screens continues (Chataway et al, 1998), the most consistent evidence of a susceptibility locus appears to be the HLA region on chromosome 6. It seems unlikely that any other single genes contribute a significant risk.

Genetic factors may also determine disease course and severity, but HLA polymorphisms are not significant contributors (Weinshenker et al, 1998). Polymorphisms in the interleukin-1 β -receptor and interleukin-1 β -receptor antagonist genes (Schrijver et al, 1999), the apolipoprotein E gene (Evangelou et al, 1999), and immunoglobulin Fc receptor genes (Myrh et al, 1999) have been associated with disease course. These associations require confirmation.

Pathophysiological Features of Multiple Sclerosis

The pathological signature of MS is the white matter plaque, a circumscribed area of demyelination and relative axonal preservation. Plaques may occur anywhere within the white matter but favor the periventricular regions, optic nerves, brain stem, cerebellum, and spinal cord. Depending on their stage of development, they contain varying proportions of immune cells and immunoreactive substances. We review current knowledge for several questions concerning immune cell recruitment and entry into the CNS, initiation and propagation of active lesions, and the mechanisms and patterns of demyelination, axonal injury, remyelination, and cell loss.

What Is the Composition of the MS Plaque?

Multiple sclerosis plaques may be characterized as active or inactive (Lassmann et al, 1998). There are several methods for determining plaque activity, but the most dependable seems to be the presence in macrophages of specific myelin degradation products (reactive for myelin basic protein [MBP], myelin oligodendrocyte glycoprotein [MOG], and proteolipid protein [PLP]) and activation markers (including MRP 14 and 27E10) (Brück et al, 1995; Lucchinetti et al, 2000a). Macrophages are especially plentiful in active

lesions (Lassmann et al, 1998), which are hypercellular and contain patchy infiltrates of autoreactive T cells and antigen-nonspecific monocytes and macrophages within a zone of myelin loss (Fig. 1). Macrophages and lymphocytes form prominent perivascular cuffs and invade the parenchyma, whereas plasma cells and B cells tend to concentrate in the perivascular region only (Prineas and Wright 1978). Most lymphocytes within plaques are T cells, including both CD4+ (helper) and CD8+ (cytotoxic) cells; conflicting data exist concerning their relative proportions (Raine, 1994) (see "Direct Cell-Mediated Injury"). The CD4+ cells can be functionally divided into Th1 (secretion of "proinflammatory" cytokines, such as tumor necrosis factor- α [TNF- α] and gamma-interferon [γ -IFN]) or Th2 (secretion of interleukins [IL]-4, -5, -6, and others) phenotypes; the relative proportions of these cells and their activity levels may contribute to lesional activity. Reactive astrocytes are usually present in the periphery of the lesion.

Actively demyelinating plaques may conform to one of four postulated distinct pathological patterns (Lucchinetti et al, 2000a). In patterns I and II, macrophages and T cells predominate in well-demarcated plaques that surround small veins and venules; pattern II is distinguished by the local precipitation of immunoglobulin (primarily IgG) and activated complement in regions of active myelin damage. In both patterns, the expression of all myelin proteins (eg, MBP, PLP, MOG, and myelin-associated glycoprotein [MAG]) are reduced to similar degrees, and oligodendrocytes are variably lost at the plaque edge, with reappearance of oligodendrocytes within the plaque center (Fig. 2a). Remyelination is extensive in lesion patterns I and II.

Pattern III lesions also contain a cellular infiltrate mainly composed of macrophages, T cells, and activated microglia. These ill-defined plaques are not vessel-centered. Immunoglobulin and complement deposition are absent; however, there is a preferential loss of MAG compared to the other myelin proteins. This pattern is associated with severe oligodendrocyte loss and evidence of oligodendrocyte apoptosis (Fig. 2b). Pattern IV also demonstrates macrophage and T cell inflammation without immunoglobulin or complement staining, but with nonapoptotic oligodendroglial death in the normal-appearing periplaque white matter and loss of all myelin proteins at the active edge of the plaque. Remyelination is minimal in pattern III and IV lesions, and each suggests a primary injury to the oligodendrocyte. These conclusions are supported by ultrastructural studies of stereotactic brain biopsies from MS patients, which revealed a group of lesions demonstrating primary alterations in the most distal oligodendrocyte processes ("distal, dying-back oligodendroglialopathy") (Rodriguez and Scheithauer, 1994). In autopsy cases studied thus far, all active lesions from an individual patient conform to a single immunopathological pattern.

Patients with chronic MS have few active plaques. Chronic plaques display well-demarcated areas of hypocellularity with myelin pallor or loss (Fig. 3). There are varying degrees of axonal loss, usually most obvious in the lesional center (Barnes et al, 1991; Raine, 1991). There is typically a persistent but minor inflammatory response, with only a few scattered perivascular lymphocytes present, although plasma cells may occasionally be prominent (Prineas and

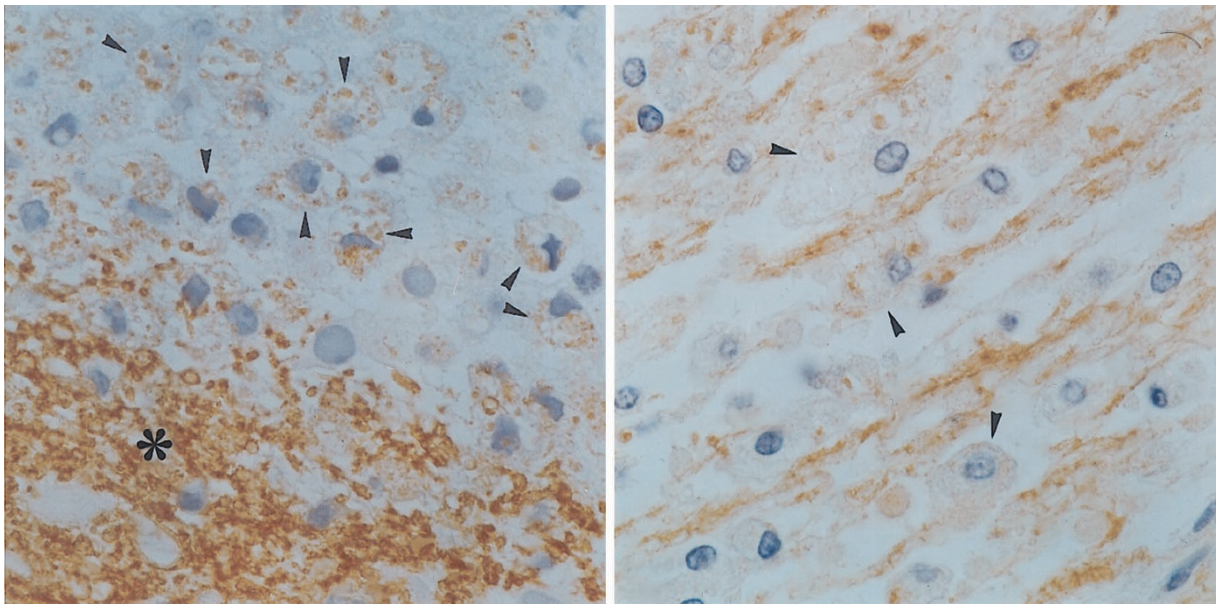


Figure 1.

Photomicrographs of an actively demyelinating multiple sclerosis lesion (immunocytochemical staining of myelin oligodendrocyte glycoprotein [brown] with hematoxylin counterstaining of nuclei [blue]). Left panel, At the active edge of a multiple sclerosis lesion (indicated by the asterisk), the products of myelin degradation are present in numerous macrophages (arrowheads). Right panel, Macrophages containing myelin debris (arrowheads) are interdigitated with degenerating myelin sheaths. (Both panels, Magnification, $\times 100$.) (Reprinted from Noseworthy et al *N Engl J Med* 2000;343:938–952. Copyright © 2000 Massachusetts Medical Society. All rights reserved.)

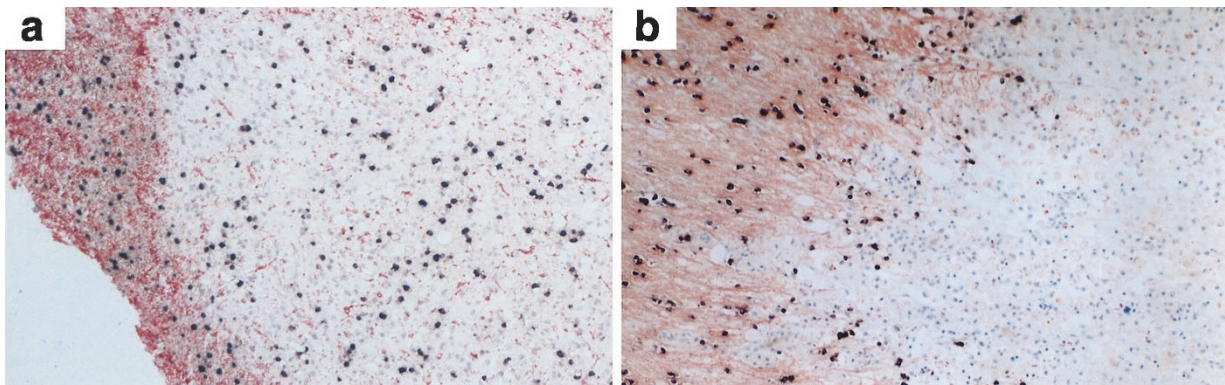


Figure 2.

Photomicrographs of oligodendrocyte preservation and loss in multiple sclerosis (MS). Panel a, Oligodendrocyte preservation. Many oligodendrocytes are seen adjacent to and in the center of a zone of active demyelination (in situ hybridization for proteolipid [PLP] mRNA [black] and immunocytochemistry for PLP protein [red]). Panel b, Oligodendrocyte loss. In a second case, oligodendrocytes are absent from a zone of active demyelination but are preserved in the adjacent periplaque white matter. (Reprinted by permission from *Nature* Supplement 399: A45 copyright 1999, Macmillan Magazines Ltd.)

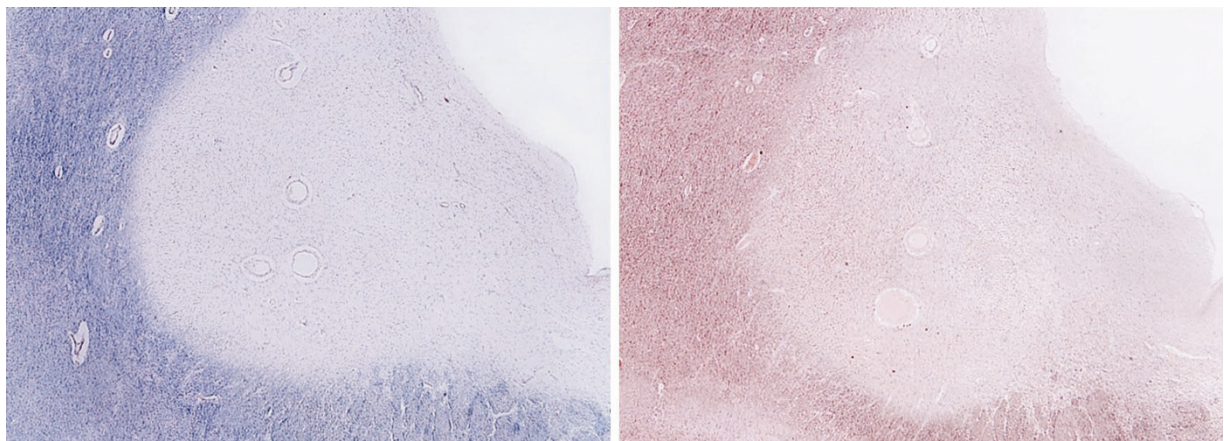


Figure 3.

Photomicrographs of a chronic multiple sclerosis plaque. In left panel, a well-demarcated hypocellular region of myelin loss is evident in the periventricular white matter (Luxol fast blue and periodic acid-Schiff myelin stain, $\times 15$ magnification). In right panel, neurofilament staining for axons in the same lesion demonstrates a reduction in axonal density. (Reprinted from Noseworthy et al, *N Engl J Med* 2000, 343:938–952. Copyright © 2000, Massachusetts Medical Society. All rights reserved.)

Wright, 1978). There are few or no oligodendrocytes, but there may be sizeable numbers of oligodendrocyte precursor cells (Wolswijk, 1998).

Shadow plaques are circumscribed regions where axons maintain uniformly thin myelin sheaths; they may occur within acute plaques or at the edge of chronic ones (Fig. 4). These plaques represent areas of remyelination and are macroscopic evidence that the CNS white matter possesses the means for self-repair. Shadow plaques are seen in conjunction with actively demyelinating lesions that retain viable oligodendrocytes in the plaque center (patterns I and II).

The next four sections consider questions that concern the inflammatory mechanisms postulated to lead to plaque development in patterns I and II outlined above. The potential processes that are operative in determining type III and IV pathological patterns are discussed in the sections on tissue injury mechanisms, axonal loss, and non-autoimmune processes that result in cell death.

How Do the Constituent Cells of a Plaque Enter the CNS in Immune-Mediated Models of Inflammatory Demyelination?

An intact blood-brain barrier allows limited passage of T lymphocytes that may not have antigen specificity. This may be initiated by the interaction of adhesion molecules expressed on the surface of lymphocytes with complementary integrins present on the endothelium, resulting in T cell rolling and adherence to the luminal surface (Fig. 5). Examples of such molecules include vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM), each expressed on endothelial cells; and very late antigen 4 (VLA-4; also called $\alpha 4$ -integrin) and lymphocyte function-associated antigen-1 (LFA-1), each displayed by T lymphocytes. Various selectins are also involved. Rolling, adherence, and diapedesis of T lymphocytes are modulated by VCAM/VLA-4 and ICAM/LFA-1 interactions.

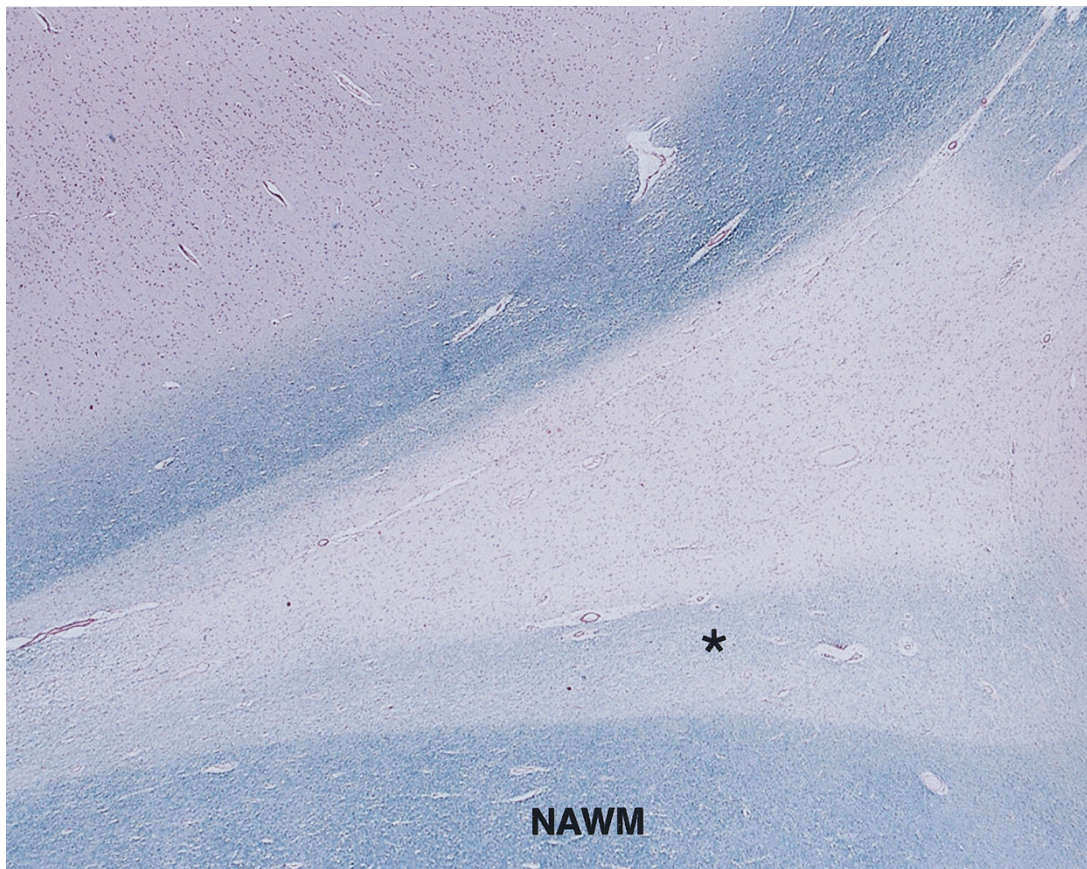


Figure 4.

Remyelination in a lesion associated with chronic multiple sclerosis. The area stained pale blue (*asterisk*) represents a region of partial remyelination (a shadow plaque) along the periventricular edge of a lesion in a patient with chronic multiple sclerosis (Luxol fast blue and periodic acid-Schiff myelin stain, $\times 15$ magnification). *NAWM* denotes normal-appearing white matter. (Reprinted from Noseworthy et al, *N Engl J Med* 2000, 343:938–952. Copyright © 2000 Massachusetts Medical Society. All rights reserved.)

Immunochemical studies and gadolinium-enhanced brain magnetic resonance imaging (MRI) findings (Filippi et al, 1996) indicate that the blood-brain barrier is disrupted in MS and experimental allergic encephalomyelitis (EAE), a putative animal model of the disease. This disruption is present primarily in active lesions, but also to a lesser degree in apparently inactive chronic plaques. Disruption and inflammation of the barrier facilitates the passage of potentially pathogenic cells and antibodies into the CNS (Wisniewski and Lossinsky, 1991; Archelos et al, 1999). The mechanism by which the barrier is disrupted is not known, but immune interactions are likely the main contributors. Interferon-gamma ($\text{IFN-}\gamma$) and tumor necrosis factor alpha ($\text{TNF-}\alpha$), major inflammatory cytokines expressed in MS lesions, can induce endothelial cells to express VCAM and major histocompatibility complex (MHC) class II molecules. Viral infection (which often precedes clinical exacerbations), the presence of bacterial antigens or superantigens, and environmental factors such as reactive metabolites and metabolic stress may also induce such changes.

In EAE, activated T-lymphocytes may use P-selectin to enter the CNS very early in the disease process before the barrier becomes inflamed (Carrithers et al, 2000). Molecules such as VCAM, ICAM, VLA-4, and

LFA-1 do not appear to have a role in early T cell entry (Baron et al, 1993; Steffen et al, 1994). However, once the barrier is inflamed, VCAM/VLA-4 and ICAM/LFA-1 interactions, in conjunction with other factors such as CD4-MHC class II binding, allow autoreactive T cell diapedesis and entry into the CNS (Archelos et al, 1993; Baron et al, 1993; Engelhardt et al, 1997; Romanic et al, 1997; Steffen et al, 1994). P-selectin does not appear to have a role in later EAE stages (Engelhardt et al, 1997). Inhibition of VLA-4 reverses clinical paralysis in acute EAE and prevents relapses in the chronic form of the disease (Yednock et al, 1992).

In human MS lesions, integrins are expressed on inflamed endothelial cells, T cells, and neural cells (microglia, oligodendrocytes, and astrocytes) and play important roles in developing and maintaining the plaque (Archelos et al, 1999; Cannella and Raine, 1995). Circulating levels of ICAM-1 and VCAM-1 are elevated in RRMS, and the profile may differ from PPMS, a finding that may allow further dissection of the differing pathophysiology of these forms of MS (Durán et al, 1999; Giovannoni et al, 1997; McDonnell et al, 1999). There are down-regulatory systems that probably control the extent of inflammation. For example, $\text{TNF-}\alpha$ -induced VCAM-1 expression may be followed by release of soluble VCAM-1, which may



Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.