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BIOLOGY OF DISEASE

Multiple Sclerosis: Current Pathophysiological Concepts

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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 oligodendrogliopathy" 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

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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-1β-receptor and interleukin-1β-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-alpha [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 oligodendrogliopathy") (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

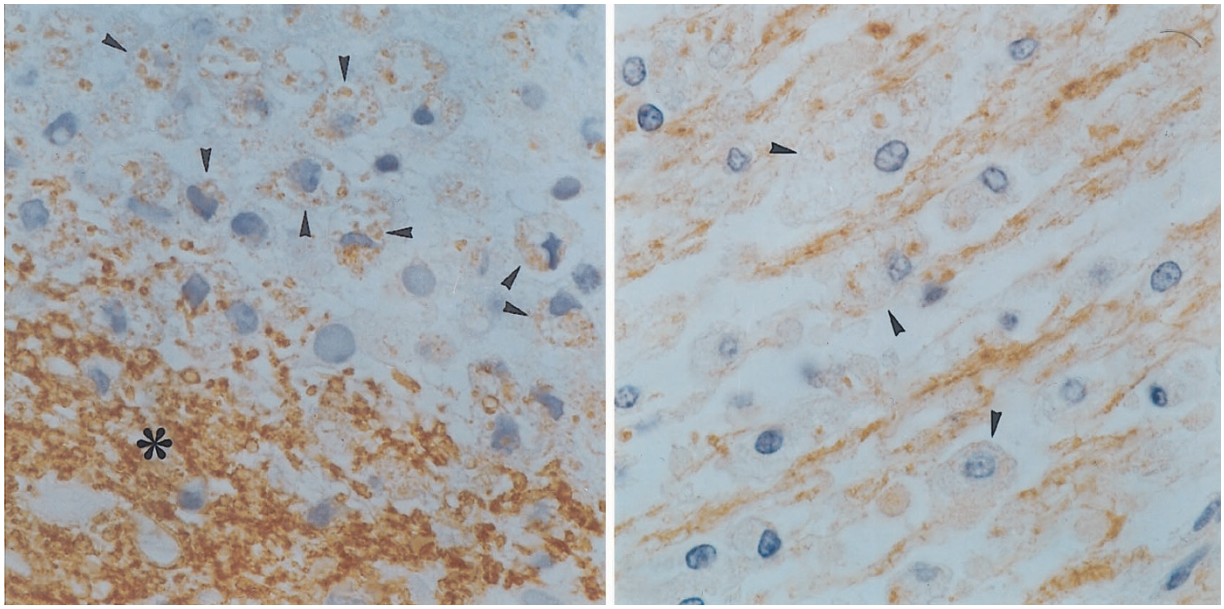

Figure 1.
Photomicrographs of an actively demyelinating multiple sclerosis lesion (immunocytochemical staining of myelin oligodendrocyte glycoprotein [brown] with hematoxylin counterstaining of nuclei [blue]). Left panel, At the active edge of a multiple sclerosis lesion (indicated by the *asterisk*), 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, ×100.) (Reprinted from Noseworthy et al N Engl J Med 2000;343:938–952. Copyright © 2000 Massachusetts Medical Society. All rights reserved.)

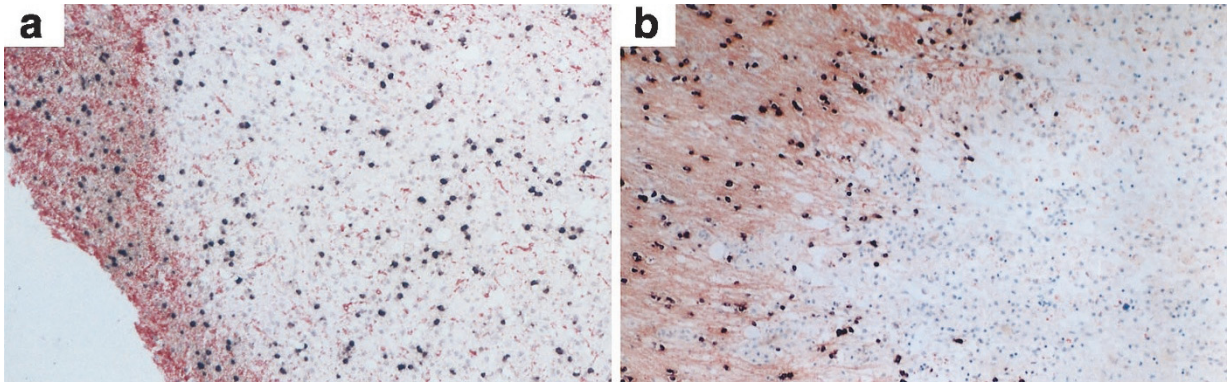

Figure 2.
Photomicrographs of oligodendrocyte preservation and loss in multiple sclerosis (MS). Panel a, Oligodendrocyte preservation. Many oligodendrocytes are seen adjacent to and in the center of a zone of active demyelination (in situ hybridization for proteolipid [PLP] mRNA [black] and immunocytochemistry 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.)

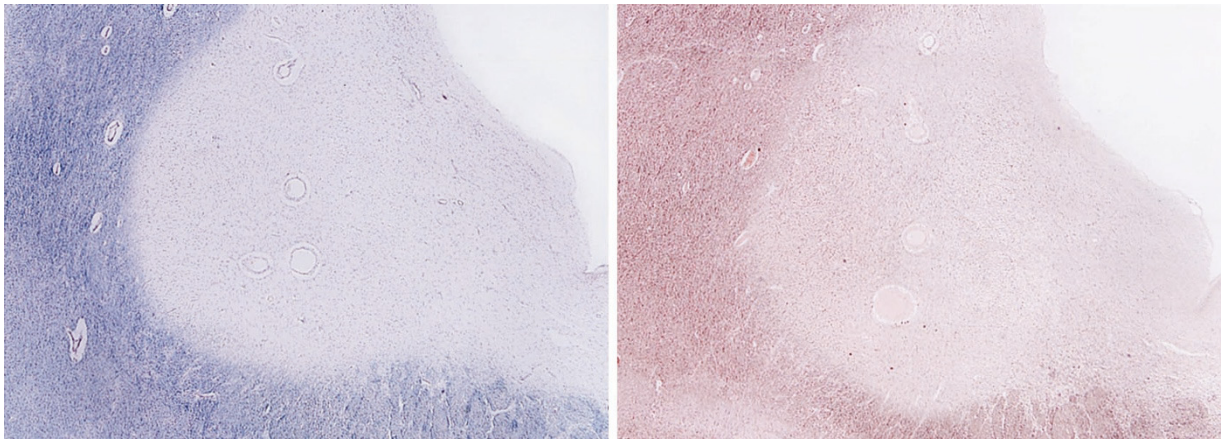

Figure 3.
Photomicrographs of a chronic multiple sclerosis plaque. In left panel, a well-demarcated hypocellular region of myelin loss is evident in the periventricular white matter (Luxol fast blue and periodic acid-Schiff myelin stain, ×15 magnification). In right panel, neurofilament staining for 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 α4-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.

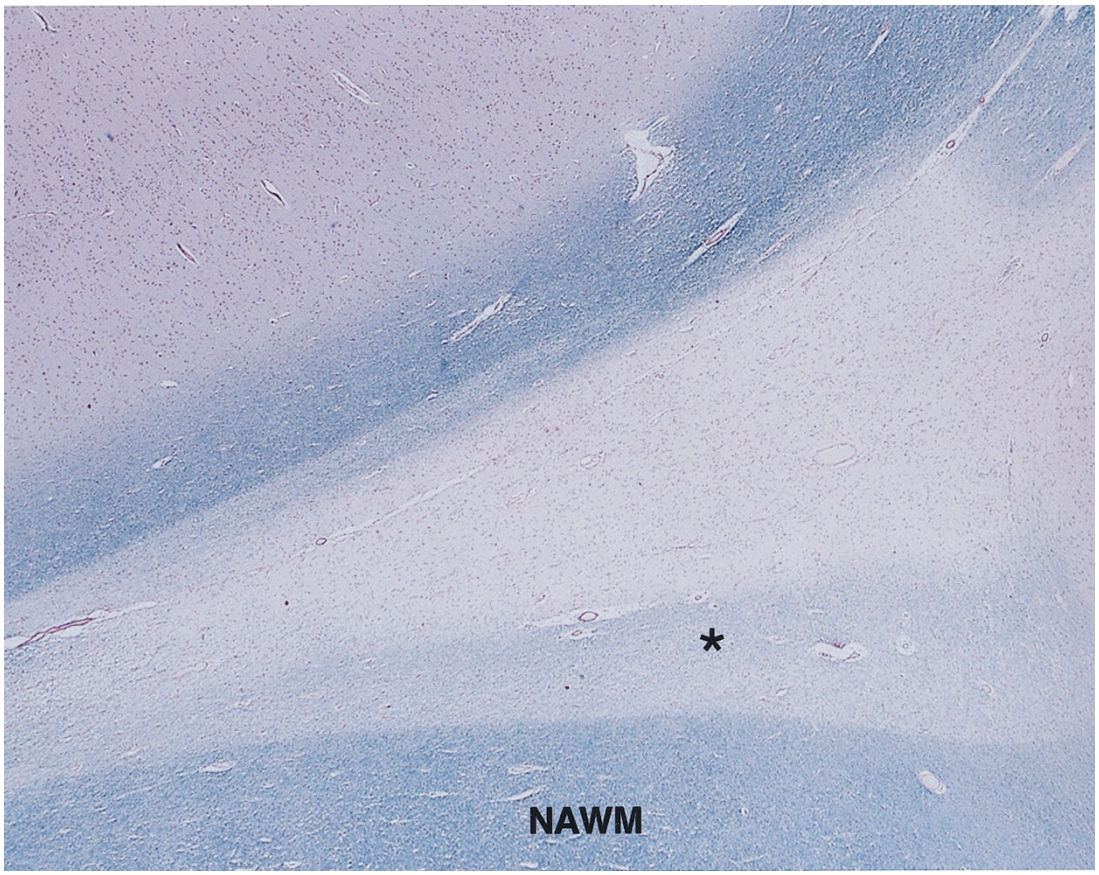

Figure 4.
Remyelination in a lesion associated with chronic multiple sclerosis. The area stained pale blue (*asterisk*) represents a region of partial remyelination (a shadow plaque) along the periventricular edge of a lesion in a patient with chronic multiple sclerosis (Luxol fast blue and periodic acid-Schiff myelin stain, ×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 (IFN-γ) and tumor necrosis factor alpha (TNF-α), 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, TNF-α-induced VCAM-1 expression may be followed by release of soluble VCAM-1, which may

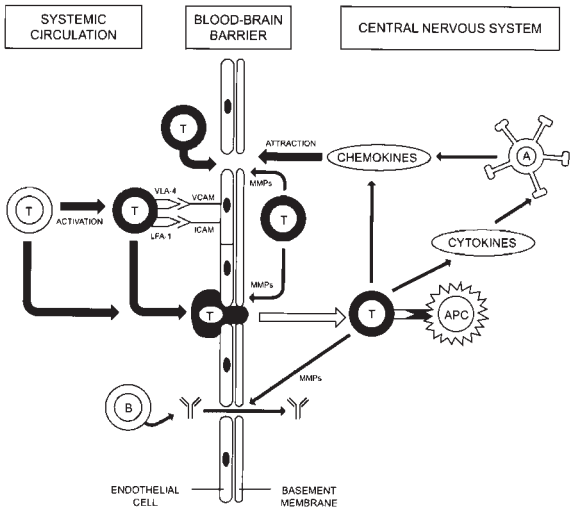

Figure 5.

Schematic of the initiation, entry, and subsequent recruitment of immune cells into the central nervous system. Both activated and nonactivated T lymphocytes may pass through the intact blood-brain barrier assisted by selectins, but this process is facilitated by barrier disruption or inflammation perhaps triggered by viral infection or reactive metabolites, among other causes. Barrier injury up-regulates adhesion molecules on the endothelial surface causing more immune cells to roll, adhere, and diapedese into the CNS. Upon entry, they produce matrix metalloproteinases (MMPs), which degrade the extracellular matrix and further disrupt the blood-brain barrier allowing easier passage of immune cells and antibodies secreted by B lymphocytes. Following formation of a co-stimulated trimolecular complex, the T lymphocyte is (re)activated and secretes cytokines that cause surrounding immune cells and glia to produce chemokines. These chemoattractant substances recruit more cells into the CNS, amplifying the inflammatory response.

block adhesion and limit cellular infiltration (Kallmann et al, 2000). The control of these systems is only partially understood. Modulation of adhesion molecule interactions is a logical strategy for MS preventative therapies.

How Does the Plaque Develop in Immune-Mediated Models of Inflammatory Demyelination?

Once autoreactive T cells have gained entry into the CNS, they invade the extracellular matrix aided by their secretion of matrix metalloproteinases (MMPs), especially MMP-9 (gelatinase B) (Yong et al, 1998b). These enzymes degrade the type IV collagen matrix and are involved in proteolysis of myelin components and regulation of cytokine production, including TNF-α (Chandler et al, 1997). MMPs facilitate conversion of a number of important pro-molecules that require proteolytic processing to their active forms (cytokines [eg, pro-TNF-α or -β], cytokine receptors [eg, IL-6 receptor-α] and adhesion molecules [eg, VCAM, L-selectin]) and regulate growth factor bioavailability (eg, releasing fibroblast growth factors from cell surfaces) (Izumi et al, 1998; Whitelock et al, 1996; Yu and Stamenkovic, 2000). MMPs also play a role in regulating apoptotic cell death by disrupting cell-matrix contacts with the subsequent loss of integrin signaling (anoikis) (Alexander et al, 1996; Chen and Strickland, 1997). Tissue inhibitors of MMPs (TIMPs) regulate MMP activity by forming molecular complexes with them.

Several lines of evidence suggest that MMPs have a central role in lesion formation in EAE and MS. MMP-9 expression in the CSF of animals with EAE correlates with clinical severity (Clements et al, 1997). Inhibitors of MMP reduce EAE disease activity and severity (Gijbels et al, 1994; Liedtke et al, 1998), and immunization with myelin antigens failed to induce EAE in young MMP-9 genetic knockout mice (Dubois et al, 1999). MMPs and TIMPs are present in the serum and CSF of MS patients and expressed in plaques (Cuzner et al, 1996; Maeda and Sobel, 1996). Serum MMP-9 and TIMP-1 and TIMP-2 levels are elevated, MMP-9 levels may be higher during relapses than periods of clinical inactivity, and MMP-9 levels may correlate with brain MRI markers of inflammation (Lee et al, 1999). Other studies have found elevated CSF MMP-9 levels in RRMS irrespective of clinical activity and without TIMP up-regulation (Leppert et al, 1998). Beta interferons, which reduce exacerbation frequency and severity in MS, are potent MMP 9 inhibitors (Yong et al, 1998a). These therapies may act in part by limiting T cell infiltration and modulating the production of demyelinating cytokines (Uhm et al, 1999). There are theoretical disadvantages to these inhibition strategies, including unknown effects on the MMP activities that regulate cytokines and apoptosis.

The initial invasion of T cells into the CNS is followed by recruitment and attraction of secondary inflammatory cells such as macrophages. Tissue injury and demyelination is thought to occur as a result of T cell attraction and activation of these cells. Integrins and chemokines mediate this recruitment process.

Chemokines are a superfamily of chemoattractant cytokines that are secreted by leukocytes and many other cell types (Luster, 1998). The major subfamilies are C-X-C (or α family) and C-C (β family); more than 40 chemokines and 10 receptors have been described so far. Members of the α family attract neutrophils (eg, IL-8) or activated T cells (eg, interferon inducible protein 10 [IP-10]). The β family seems to mediate chronic inflammation and individual members are attractants for monocytes, T cells, or eosinophils. Selective expression of individual chemokines may influence the cellular composition of inflammatory lesions, because some chemokine receptors are associated with either Th1 or Th2 responses. Th1 proinflammatory cells may be associated with CCR5 (receptors for the chemokines RANTES, MIP-1α, and MIP-1β) and CXCR3 (receptors for IP-10 and MIG). In contrast, Th2 anti-inflammatory cells may shift towards display of CCR3 (receptors for MCP-3, MCP-4, and RANTES), CCR4 (receptors for TARC and MDC), and CCR8 (Bonecchi et al, 1998; Sallusto et al, 1998). In MS, some chemokine receptors, including CCR5 and CXCR3, may be overexpressed in peripheral and lesional T lymphocytes (Zhang et al, 2000), and CSF may contain elevated levels of the chemokines IP-10, RANTES, and MIG (Sorensen et al, 1999). The specific chemokine profiles of importance in MS are unknown, but there may be preferential peripheral T cell migra-

tory pattern toward RANTES and MIP-1α in association with up-regulation of their CCR5 receptor (Zang et al, 2000).

Which Antigens Do Autoreactive T Cells Target in Immune-Mediated Models of Inflammatory Demyelination?

Immune dyscontrol, perhaps involving tolerance mechanisms, may contribute to the initiating or propagating of a pathological state by autoreactive T cells. The causative autoantigen(s) in MS are still not known, but autoreactive T cells exist in both healthy control subjects and people with MS. The leading candidates are myelin protein constituents. The role of MBP-reactive T cells is the most thoroughly studied. Data concerning the frequency of MBP-specific autoreactive cells in MS versus controls are conflicting, but their level of activation may be greater in MS (Martino and Hartung, 1999). On the other hand, many different ligands can induce highly variable responses from single anti-MBP T cells, indicating a high degree of degeneracy in antigen recognition and challenging the role of MBP as a primary autoantigen (Hemmer et al, 1998; Vergelli et al, 1997). The roles of the other myelin components, PLP, MOG, and MAG, and their autoreactive T lymphocyte counterparts is less well studied. Many other putative autoantigens have been implicated in MS, also with respect to antibody-mediated responses (see below).

Molecular mimicry (similarity among antigens contained in microbes and human tissue) has been hypothesized to explain immunological injury in autoimmune diseases (Brocke et al, 1998). Under this schema, antigens present in or originating from an exogenous pathogen activate T cells; these cells then induce CNS demyelination by recognizing cross-reactive myelin antigens. This explanation has been used to implicate HHV-6 in MS pathogenesis (Steinman and Oldstone, 1997) although a latent viral infection, rather than mimicry, could also potentially result in demyelination and oligodendroglial loss. The T cell receptor normally maintains an extremely high level of cross-reactivity, probably to balance the requirement to recognize non-self antigens and to reduce the possibility of loss of self-tolerance (Mason, 1998). The concept of molecular mimicry remains speculative.

How Do T Lymphocytes Orchestrate Processes That Cause Tissue Damage in Immune-Mediated Models of Inflammatory Demyelination?

Myelin basic protein-specific cells exist in the T cell repertoire of healthy people and those with MS. These and other autoreactive T cells may direct the initiation of the inflammatory response through blood-brain barrier disruption and accumulation of secondary inflammatory cells, principally monocytes and macrophages, which in turn mediate tissue injury. In one EAE experiment, less than 4% of perivascular inflammatory cells were antigen-specific (Cross et al, 1990) and these cells remained in the perivascular space while the parenchyma was intensely infiltrated with leukocytes. In addition, immune mechanisms involving B cells, demyelinating antibodies, and complement are required. However, there is also evidence supporting the concept that the inflammatory reaction may occur secondary to or independently from demyelination (see "Are There Factors Other Than an Autoimmune Response That Might Be Relevant to MS Pathophysiology?"). The following subsections describe processes based on primary immune-mediated models.

The Trimolecular Complex. Autoreactive T cells respond to putative MS autoantigens presented by antigen-presenting cells through formation of a trimolecular complex (Fig. 6). Perivascular monocytes, microglia and macrophages, parenchymal lymphocytes, and possibly astrocytes express MHC antigens in MS. There are two principal types of MHC molecules: class I (includes HLA-A, -B, and -C) and class II (includes HLA-DR, -DP, and -DQ). These molecules bind peptide antigens as part of the "processing" they require for presentation to different T lymphocytes. Lymphocytes of the CD4+ type recognize antigens in con-

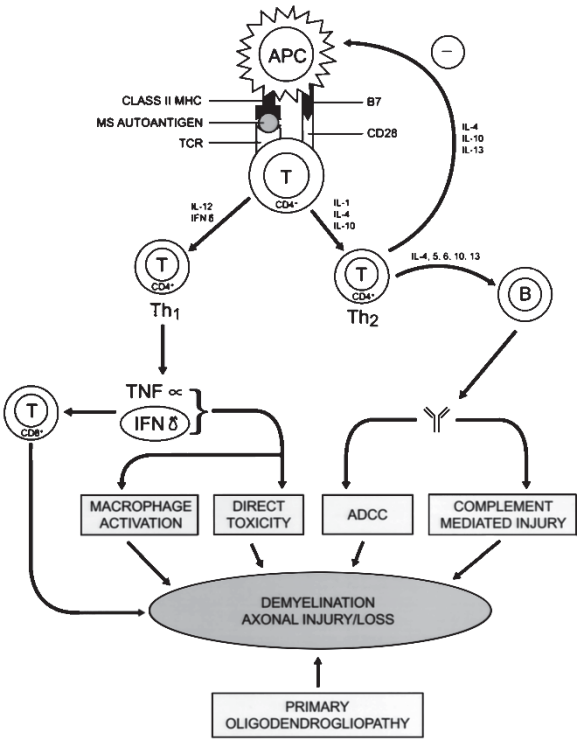

Figure 6.
Schema of autoantigen-induced T cell activation and orchestration of the immune pathways leading to demyelination and axonal loss. Once in the CNS, CD4+ T lymphocytes interact with antigen-presenting cells (APC) via the trimolecular complex (MHC class II molecules, the T cell receptor [TCR] and the inciting autoantigen). In the presence of B7-1 and B7-2 co-stimulatory ligands, the complex triggers specific response that depends on the T cell co-stimulatory molecules (eg, CD28 = immune response; CTLA-4 = anergy) and the cytokine milieu (IL-12 leads to Th1 differentiation; IL-4 leads to Th2 phenotype). Th1 cells secrete TNFα and IFNγ, each of which activates phagocytic macrophages and are toxic to myelin and oligodendrocytes. IFNγ may also stimulate production of MHC class I-restricted CD8+ T lymphocytes that are directly cytotoxic. The Th2-type cells produce interleukins that result in antibody production and complement deposition. The final pathway of demyelination and axonal loss may incorporate some or all of these mechanisms in conjunction with processes (persistent viral infection; toxins) that lead to primary oligodendrocyte loss.

junction with MHC class II molecules, whereas CD8+ lymphocytes recognize antigens in the context of MHC class I molecules. The trimolecular complex is completed by interaction with the T cell receptor.

Multiple sclerosis seems primarily to be a disease involving immune responses to antigens presented by class II molecules, although class I mechanisms are receiving increased attention. The interaction between a CD4+ T lymphocyte and an antigen-presenting cell (APC) results in antigen-specific signaling, but T cell activation requires the presence of co-stimulatory molecules. Co-stimulatory molecules CD28 and CTLA-4 are present on the T cell surface. These molecules interact with their ligands B7-1 and B7-2 to promote activation; when absent, the T cell-APC interaction results in T cell apoptosis and limitation of the immune response (Anderson et al, 1999). When the co-stimulatory molecules and their respective ligands are present, intracellular signaling pathways involving phosphorylation-dependent enzymes and second messenger systems activate secretory and proliferative mechanisms within the T lymphocyte. Once activated, T cells can then orchestrate several cellular and humoral responses that result in tissue injury.

Cytokine-Mediated Immune Responses: Th1 and Th2. Activated T-helper cell subsets have distinct profiles of cytokine release that regulate humoral immune responses. The oversimplified schematic that describes a functionally balanced dichotomy of Th1 and Th2 lymphocytes (Abbas et al, 1996; Romagnani, 1997) remains useful and remains a model of MS pathogenesis (Fig. 6). Under this schema, Th1 lymphocytes and their proinflammatory products (including IL-2, IFN-γ, and TNF-α) seem to play a primary role in initiation and/or propagation of autoimmune diseases, including the inflammatory MS lesion. Th2 cells secrete cytokines such as IL-4, -5, -6, -10, and -13, which promote B cell antibody production and allergic responses. The development of a Th1 or Th2 response may depend on the cytokine milieu present during initial and secondary antigen presentation as well as the types of peptides and co-stimulatory molecules involved (Constant and Bottomly, 1997; O'Garra, 1998). As demonstrated in Figure 6, IL-12 and IFN-γ presence selectively promotes a Th1 phenotype with subsequent CD4+ T cell production of IFN-γ, whereas a milieu containing an abundance of IL-1, -4, and -10 will favor the secretion of Th2-pattern interleukins. Other regulatory T cells, perhaps of the Th3 phenotype, may protect against development of EAE. These cells may express different T-cell receptor V-beta chains than the T lymphocytes that initiate the disease (Olivares-Villagomez et al, 1998; 2000).

The Th1 response pattern appears to direct disease activity in several autoimmune animal models, including EAE, and a shift towards Th2 predominance is associated with an improved disease course. There is only indirect evidence that Th1 cytokines and their effects drive the pathophysiology of MS. Signature Th1 and Th2 cytokines are present in MS lesions and CSF (Cannella and Raine, 1995; Navikas and Link, 1996). Plaques contain TNF-α and IFN-γ (Selmaj et al, 1991b); within actively demyelinating plaques, these cytokines are expressed in the invading leukocytes and some local glial cells. Levels of TNF-α and IFN-γ may also be elevated during relapses (Beck et al, 1988). Treatment with IFN-γ may trigger clinical relapses in MS (Panitch et al, 1987). The spectrum of chemokines and their receptors is consistent with a Th1-driven response. Finally, there are pathological similarities between MS and EAE, a model of Th1-mediated autoimmunity.

There is increasing recognition that the Th1-driven MS model explains only some of the immunological and pathological features of MS. Exceptions to Th1 exclusivity include the following: CD8+/MHC class I-restricted T lymphocytes often outnumber CD4+ lymphocytes, especially at the sites of tissue destruction in active lesions (whereas the CD4+ phenotype predominates in the perivascular space); clonal expansion in active lesions may be much more prominent in the CD8+ population (Babbe et al, 2000); the extent of axonal injury and tissue destruction may better correlate with the number of macrophages and CD8+ cells than with CD4+ cells (Bitsch et al, 2000); some fulminant lesions contain an abundance of granulocytes and eosinophils (a finding not compatible with a pure Th1 disease) (Lucchinetti et al, 2000b); and an opticospinal inflammatory reaction similar to Devic's syndrome can be induced in animals by transfer of Th2-polarized autoreactive T lymphocytes (Lafaille et al, 1997). These facts suggest that the pathophysiological processes that underlie lesion development in MS are more complex than can be explained by an exclusively Th1-directed process and that other factors, both immune and nonimmune, may play significant roles in initiating and propagating the disease process.

What Are the Immune Mechanisms of Tissue Injury?

Current data suggest that the inflammatory component of the MS lesion may be regulated by T lymphocytes but that additional mechanisms, including demyelinating antibodies and complement, are required for production of the demyelinating plaque (Kieseier et al, 1999).

The Role of Cytokines, Cytokine Receptors, and Other Soluble Mediators. In the appropriate milieu, with IL-12 and IFN-γ present, CD4+ T lymphocytes differentiate towards a Th1 phenotype and secrete TNF-α and IFN-γ. These substances can result in direct myelin injury, promote cell-mediated demyelination, and activate macrophages, astrocytes, and microglia, which in turn express TNF-α in active lesions. Interferon-γ also promotes the induction of MHC in glial cells, allowing them to potentially present antigens. Macrophages are activated by Th1-type cytokines; they are plentiful in actively demyelinating lesions and phagocytose and digest myelin constituents. Attempts to dissect the role of individual Th1 and Th2 cytokines using genetic knockout models of EAE have underscored the redundancy of the immuno-

regulatory system and provided some counterintuitive findings. For example, TNF-α administration in groups of mice, either deficient in or overexpressing TNF-α, reduced EAE severity in both groups (Liu et al, 1998). This suggests that TNF-α possesses anti-inflammatory properties under the appropriate circumstances. Chemokines, integrins, and MMPs may also be directly toxic to myelin or result in up-regulated localized immune responses (Biddison et al, 1997; Chandler et al, 1995).

Members of the TNF receptor superfamily may also promote cytotoxicity and demyelination. These molecules, which include Fas (CD95), TRAIL receptor 2, and TNF receptors p55 and p75, may generate signals to induce apoptosis, or programmed cell death. Apoptosis is a genetically controlled process of cytoskeletal disruption, membrane blebbing, and nuclear condensation that occurs under circumstances where interactions, for example between Fas ligand and the Fas receptor, trigger caspase-mediated enzymatic pathways that control cell death machinery. IFN-γ can induce Fas receptor expression on oligodendrocytes. This result may contribute to CD4+ lymphocyte-mediated oligodendrocyte destruction by Fas/Fas ligand interactions (Pouly et al, 2000). Fas-positive oligodendrocyte remnants may be detected in the phagosomes of macrophages from MS plaques (D'Souza et al, 1996). T lymphocytes in MS patients may also have defective Fas mechanisms, potentially allowing autoreactive cells to escape apoptosis and promote demyelination. These defects may be most prominent in patients with progressive disease (Comi et al, 2000). TNF receptor mechanisms also have potential roles in limitation of the immune response. Patients with MS have up-regulated Fas ligand and TRAIL receptors on circulating mononuclear cells (Huang et al, 2000); the inflammatory phase of an evolving lesion may come to an end because of Fas-regulated apoptosis of members of the invading T cell population. As with most immune regulatory mechanisms, the balance of these potentially helpful and harmful effects is of great importance.

Direct Cell-Mediated Injury. Proinflammatory cytokines may also be able to reactivate memory CD4+ T cells through two separate calcium-dependent intracellular mechanisms that allow bypass of the trimolecular complex, even in the absence of an autoantigen (Martino et al, 1998; Unutmaz et al, 1994).

Experimental evidence from various forms of animal EAE suggests that cytotoxic T cells may also have the capability of causing demyelination. As depicted in Figure 6, in some viral models of MS, certain cytokines promote CD8+ T cells to cause tissue injury; CD8+ cells are often plentiful in human MS plaques. Oligodendrocytes may be induced by cytokines to express MHC class I antigens and offer a target for a CD8+-restricted cytotoxic response, including secretion of TNF-α and IFN-γ (Grenier et al, 1989; Tsuchida et al, 1994). CD8+ lymphocytes may modulate development of neurological deficits in MHC class I-deficient mice with the Theiler's murine encephalomyelitis virus model of MS by exocytosis of granules containing perforins (Murray et al, 1998), which cause cell injury by binding to cell walls and creating lytic pores in them. There is no clear evidence that autoreactive class I-restricted CD8+ cells directly damage oligodendrocytes in MS.

Gamma-delta T lymphocytes are present in long-standing MS lesions and are toxic to oligodendrocytes in vitro (Freedman et al, 1991). Gamma-delta T lymphocytes may modulate this activity following recognition of heat shock proteins, with which they colocalize in MS plaques (Selmaj et al, 1991a). These lymphocytes secrete TNF-α and IFN-γ and may also cause oligodendrocyte injury via perforin and/or granzyme-mediated mechanisms. Granzymes are serine proteases that act in conjunction with perforins to form lytic pores in cell walls, but they also cause DNA fragmentation and directly contribute to cell death.

Antibodies and Complement. Plasma cells and B cells are detectable in MS lesions (Esiri, 1977), and cerebrospinal fluid oligoclonal banding, representing intrathecal synthesis of immunoglobulin detectable by immunoelectrophoresis, is a hallmark of the disease. Many potential autoantibodies, detected in serum and/or CSF, have been reported in MS (Archelos et al, 2000). In addition to MBP, MOG, PLP, and MAG, these include heat shock protein 60, α-B crystalline (Bajramovic et al, 2000; van Noort et al, 1995), 2',3'-cyclic nucleotide 3' phosphodiesterase, and human transaldolase, among others.

The most compelling case for a target antigen is MOG, which is a minor myelin component expressed at the outermost lamellae of the myelin sheath and on oligodendrocytes where it may be readily exposed to immune cells. In the MOG variant of EAE, anti-MOG antibodies are associated with both an encephalitogenic T cell response and demyelination (Bernard et al, 1997) along with complement activation and phagocytosis (Piddlesden et al, 1993). As such, the MOG variant of EAE more closely resembles MS than other EAE variants in which inflammation greatly predominates over demyelination. Furthermore, MOG-induced EAE produces a wide range of pathological lesions similar to that of MS, including lesional patterns of prototypic MS, optic neuritis, neuromyelitis optica, and the acute Marburg variant (Adelmann et al, 1995; Johns et al, 1995; Storch et al, 1998b). This suggests that only one autoantibody response may be required to affect the entire spectrum of MS pathology, at least on a descriptive basis. Brain MS lesions display MOG (Genain et al, 1999; Raine et al, 1999), and there may be definable subgroups of patients in whom anti-MOG antibodies are detectable (Rawes et al, 1998; Reindl et al, 1999).

The mechanisms of immunoglobulin injury have only been partially elucidated. Immunoglobulins, predominantly IgG, are deposited in regions of active plaque demyelination and where macrophages contain myelin degradation products. Receptor-mediated macrophage phagocytosis of myelin may be facilitated by antibody deposition. Antibody deposits also occur in regions where complement factors such as

C1q and C3 are present and in lesions where C9 neoantigen is colocalized to myelin degradation products within macrophages, on damaged myelin sheaths, and on oligodendrocytes (Storch et al, 1998a).

Antibodies directed against myelin constituents can cause demyelination by several means. These include antibody-mediated, cell-mediated toxicity (ADCC); release of cytokines through Fc receptor stimulation on natural killer cells, macrophages, or mast cells; myelin opsonization (promoting phagocytosis); or complement activation. More work is required to learn the pathogenic details of these events in EAE and to determine whether these mechanisms are operative in MS. Recent indirect evidence for the potential importance comes from a randomized controlled trial that demonstrated benefit for plasma exchange therapy in acute, severe CNS demyelinating syndromes (Weinshenker et al, 1999).

Other Mechanisms of Demyelination and Tissue Injury. The immune processes that occur in MS may trigger many nonimmune mechanisms that result in demyelination, axonal injury, and oligodendrocyte death. Soluble substances have been implicated in demyelination and tissue injury, including perforin (Scolding et al, 1990) and calpain, a calcium-activated neutral protease implicated in myelin protein degradation (Shields et al, 1999). The effects of reactive oxygen and nitrogen species and glutamate-driven mechanisms may also be important.

Reactive oxygen species are receiving increasing attention. Nitric oxide free radical is implicated in the modulation of inflammatory demyelination (Smith et al, 1999). Nitric oxide and the reactive substances it creates, such as peroxynitrite, are also cytotoxic, impair respiratory chain function, and can cause axonal conduction block (Redford et al, 1997; Shrager et al, 1998). Nitric oxide and peroxynitrite levels are elevated in EAE (Cross et al, 1996, 1997; Koprowski et al, 1993; Okuda et al, 1995; van der Veen et al, 1997). Nitric oxide synthase is present in active MS plaques where the inducible form of the enzyme is present in microglia and macrophages (Bagasra et al, 1995; Bö et al, 1994; De Groot et al, 1997). The effect of nitric oxide synthase inhibitors on EAE may depend on the timing of treatment. At certain stages, nitric oxide may play a protective role (Okuda et al, 1998).

Excitotoxic mechanisms have been postulated to cause irreversible cell injury in many diseases. Abnormal glutamate metabolism, mediated by AMPA/kainate glutamate receptors, is implicated in neuronal and oligodendroglial death, in part because activated immune cells produce and release large amounts of glutamate. This was studied in mouse EAE by administering the AMPA/kainate antagonist NBQX, resulting in reduced clinical symptoms, increased oligodendroglial survival and reduced axonal injury as measured by dephosphorylation of neurofilament H (Pitt et al, 2000a). These results apparently occurred independently of a primary immune effect because NBQX did not alter lesion size, the degree of inflammation, or the in vitro proliferative activity of antigen-primed T lymphocytes. These studies raise the possibility, also highlighted by the pathological studies cited previously (Lucchinetti et al, 2000a), that oligodendrocyte death/apoptosis might be independent of the degree of inflammation in MS.

What Is the Role of Axonal Damage and Loss?

It has long been recognized that axons are relatively, but not absolutely, spared in MS, especially early in the disease (Charcot, 1868). Recent pathological and noninvasive radiological studies have focused attention on how early in the disease axons may be injured or lost and the possible contribution of axonal injury to clinical disability and the development of progressive MS. Not surprisingly, axonal density is reduced in chronic plaques. Whereas estimates of axonal number and density are challenged by the variable presence of edema, myelin loss, atrophy, and inflammatory cell infiltrates, between 50% and 80% of axons may be lost in chronically demyelinated cervical spinal cord plaques (Lovas et al, 2000).

The accumulation of β-amyloid precursor protein (β-APP) identifies damaged axons in actively demyelinating MS lesions (Ferguson et al, 1997). Acute, active MS plaques may also demonstrate axonal transection, swelling, formation of terminal spheroids, and regenerative sprouting (Trapp et al, 1998). Others have confirmed early axonal loss in the early inflammatory phases of the disease (Bitsch et al, 2000; Kornek et al, 2000), even in the absence of demonstrable primary demyelination. Axonal loss is irreversible and probably underlies the worsening neurological deficits that accrue during primary and secondary progressive forms of the disease during which clinical progression correlates with brain atrophy (Losseff et al, 1996). The mechanisms of axonal injury are largely unknown. In particular, it is not clear whether inflammatory effects may damage axons directly or whether they operate primarily through a pathway that includes demyelination. Bitsch et al (2000) found that acute axonal injury correlated with the number of macrophages and CD8+ T lymphocytes within plaques but not with TNF-α or nitric oxide synthase expression. This suggests that axonal injury is not solely due to demyelination. Glutamate-driven excitotoxic mechanisms may be operative as well (Pitt et al, 2000b). A recent intriguing finding was that magnetic resonance spectroscopy could identify changes consistent with axonal dysfunction in white matter regions some distance from areas of demyelination. This may represent the wide-ranging effects of inflammation-induced white matter changes, or reorganization or alterations in axonal projection pathways (De Stefano et al, 1999).

Can Inflammation Ever Be Beneficial?

The inflammatory response in MS is generally thought to contribute in part to lesion formation although it may be either a primary or secondary phenomenon (see next section). However, the abundance of inflammation in inactive MS lesions, coupled with recent

observations demonstrating that the immune cells involved in active inflammatory responses are also paradoxically capable of neuroprotective effects, may indicate an important role for inflammation in lesion repair. Intraperitoneal administration of anti-MBP T lymphocytes seemed to protect against secondary degeneration of rat retinal ganglion cells following a crush injury of the optic nerve (Moalem et al, 1999). Macrophages may also have neuroprotective effects (Rapalino et al, 1998). T lymphocytes and other immune cells are capable of producing neurotrophic factors (Besser and Wank, 1999; Kerschensteiner et al, 1999; Torcia et al, 1996). Hohlfeld et al (2000) propose that neuroprotective effects of the inflammation may be due to immune cell production of growth factors such as glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF). Human T cells, B cells, and monocytes secrete BDNF in vitro and it is detectable in MS plaques (Kerschensteiner et al, 1999). If confirmed, these findings will have important therapeutic implications.

Are There Factors Other Than an Autoimmune Response That Might Be Relevant to MS Pathophysiology?

Immune-mediated responses may damage oligodendrocytes in plaques exhibiting pathological patterns I and II by the direct toxic and cell-mediated and humorally-mediated mechanisms reviewed above. The pathological patterns III and IV described in actively demyelinating plaques (Lucchinetti et al, 2000a) and evidence for distal oligodendroglial process atrophy (Rodriguez and Scheithauer, 1994) suggest that there are other potential mechanisms for oligodendrocyte death, resulting in a primary "dying-back" oligodendrogliopathy. The cause of this glial cell death is unknown, but potential mechanisms may include one or more of the following: persistent viral infection, local toxins, metabolic stress (ischemia), direct CD4+ and/or CD8+ T cell cytotoxicity, and abnormal cell death signaling mechanisms.

A primary T-cell driven pathophysiological process may not be applicable to all patients with MS. Neuropathological studies reveal that inflammatory cells (and specifically T cells) are not always present in areas of active demyelination (Rodriguez and Scheithauer, 1994), and persistent inflammation is a frequent and typical feature of chronic MS lesions. Gay et al (1997) recently used multifactorial cluster analysis to conclude that the bulk of T-cell infiltration into the lesions follows myelin destruction. In addition, active demyelination has been observed in immunosuppressed patients with no evidence of perivascular inflammation in the lesions (Guseo and Jellinger, 1975). Furthermore, pathological studies also suggest that inflammation may be secondary to a primary injury to the oligodendrocyte (patterns III and IV) which could result in the liberation of novel antigens triggering a secondary inflammatory response (Lucchinetti et al, 2000a; Rodriguez and Scheithauer, 1994).

What Are the Prospects for Remyelination?

Repair of myelin occurs frequently but inefficiently. Areas of thin new myelin with short internodes are restricted to the periphery of most inactive plaques. Foci of aberrant remyelination around cell processes also occur. Early in MS, more rapid myelin repair may occur, resulting in shadow plaques. The extent of repair is probably limited by the degree of oligodendrocyte survival within plaques, the presence of oligodendroglial precursor cells, or some combination of these factors, together with an appropriate milieu of cytokines, growth factors, and limitation of the underlying demyelinating process of MS. Longstanding MS lesions usually contain very few oligodendroglia (Mews et al, 1998) but may retain numerous oligodendrocyte precursor cells (Wolswijk, 1998). Acute MS lesions demonstrate tremendous heterogeneity in the degree of oligodendroglial destruction, and the extent of remyelination within a lesion is associated with the number of these cells that are spared (Lucchinetti et al, 1999).

Intuitively, efforts towards remyelination should be most effective in the setting of quiescent disease. In the Theiler's virus model of MS, immunosuppression enhances remyelination (Rodriguez and Lindsley, 1992). In the same model, "natural" monoclonal IgM autoantibodies directed against MBP and other CNS and non-CNS antigens and nonspecific polyclonal immunoglobulin administration each promote remyelination (Asakura et al, 1996; Rodriguez and Lennon, 1990; Rodriguez et al, 1996; van Engelen et al, 1994), especially if the inflammatory demyelinating process is not active. The requirement for clinically quiescent disease may also be true in humans (Noseworthy et al, 2001). The first human study of intravenous immune globulin to promote remyelination and improve motor strength was unsuccessful (Noseworthy et al, 2000b), but many variations on this therapeutic strategy remain to be explored.

Therapeutic Strategies in Multiple Sclerosis

This review of the current understanding of MS pathophysiology suggests many potential targets and modes of therapy for MS (Table 1). Interventions aimed at preventing cell-cell and cell-endothelial signaling through interference in adhesion molecule and chemokine mechanisms may reduce immune cell penetration into the CNS and inhibit recruitment of the secondary cells that mediate tissue injury. The upregulation of adhesion molecules and chemokines that has been demonstrated in MS provides an inviting target for therapeutic antiadhesion molecule antibodies and antichemokines. Inhibition of MMPs may reduce the degree of parenchymal invasion by autoreactive T lymphocytes and other immune cells and may favorably alter the local cytokine milieu. Interference with the trimolecular complex and antigen recognition provides several possible interventions. Targets of immunotherapy may include the MHC complex or T cells (each via monoclonal antibodies or vaccines),

Table 1. Therapeutic Approaches Based on Putative Pathophysiological Mechanisms in Multiple Sclerosis

Process	Mediators	Therapeutic approach
Cell entry rolling	L-, E-, P-selectins	Selectin inhibitors
		Anti-selectin MAbs
Adherence, Diapedesis	L-, E-, P-selectins	
	Integrins	
	VLA-4, VLA-5, VLA-6	Selectin inhibitors
	LFA-1, MAC-1, CR4	Anti-selectin MAbs
	Ig superfamily	Integrin/ligand inhibitors
	ICAM-1, ICAM-2, ICAM-3	Anti-adhesion molecule MAbs
	VCAM, LFA-2, LFA-3	
BBB Disruption	? Viral infection	Antiviral therapies
	? Reactive metabolites	Free radical scavengers
		Nitric oxide synthase inhibitors
	Cytokines	Anticytokine drugs/cytokines/MAbs
	MMP	MMP inhibitors
		Anti-MMP MAbs
Cellular CNS Invasion	MMPs	MMP inhibitors/Anti-MMP MAbs
	TIMPs	TIMP promoters
Cell recruitment	Integrins	Integrin inhibitors
		Anti-integrin MAbs
	Chemokines	Chemokine inhibitors
		Anti-chemokine MAbs
		Cytokine manipulation
Trimolecular complex	Class II MHC	Anti-MHC MAbs
		Hypervariable peptide vaccines
	TCR	TCR vaccines
		Altered peptide ligands
	B7-1, B7-2	Anti-B7 MAbs
	T cell ligands	CD/40/CD40 ligand interactions
		CTLA-4 Ig fusion protein
	T cell antigens (CDs)	Anti-T cell MAbs
		T cell vaccines
	Adhesion molecules	Anti-adhesion molecule MAbs
Immune amplification	Th1 predominance	Anti-Th1 cytokines
		Th1 cytokine inhibitors
		Th2 cytokines (II-4, IL-10, TGFβ)
		Th2 cytokine promoters
		Shift Th1 to Th2 (drugs, etc.)
Direct toxicity	TNFα, IFNγ, lymphotoxin	Anti-cytokine MAbs
		Cytokine inhibitors
	Proteases/MMPs	Anti-proteases/Anti-MMP MAbs
		TIMP promoters
Cell-mediated injury	Macrophages	General/specific immunosuppression
	Microglia	Cell-specific MAbs
	CD4+/CD8+ T cells	Autologous stem-cell transplantation
Humorally-mediated injury	B cells	Immunosuppression
		Cell-specific MAbs
		Autologous stem-cell transplantation
	Antibodies	Anti-autoantibody MAbs
		Polyclonal immunoglobulins
		Plasmapheresis
	Complement	Complement/Membrane attack complex inhibitors (CD59, CD46, decay-accelerating factor [DAF])
		Complement MAbs
Other injury mechanisms	Reactive oxygen species	Free radical scavengers
	Reactive nitrogen species	Nitric oxide synthase inhibitors
	Glutamate	AMPA/kainite inhibitors
	Apoptosis	Fas/Fas-ligand and other receptor modulation
	Viral infection	Antiviral therapies
	Bacterial infection	Antibiotics

Table 1. Continued

Process	Mediators	Therapeutic approach
Remyelination	Oligodendrocyte precursor availability	Precursor cell implantation Stem cell transplantation Immortalized cell transplantation Schwann cell transplantation Xenotransplantation
	Oligodendrocyte precursor survival/maturity	Brain-derived growth factor Glial cell line-derived growth factor Insulin-like growth factor-1 IL-6 Leukemia inhibitory factor Neurotrophin-3 Platelet-derived growth factor Ciliary neurotrophic factor
Any implicated process	Any mechanism	Gene therapy

BBB, blood-brain barrier; VLA, very late antigen; LFA, lymphocyte function-associated antigen; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of MMP; MHC, major histocompatibility complex; TNFα, tumor necrosis factor α; IFNγ, interferon γ; MAb, monoclonal antibody.

altered peptide ligands, and co-stimulatory molecules. Anticytokine strategies may lead to multifaceted approaches aimed at shifting the T lymphocyte balance towards Th2 predominance by the use of drugs or techniques that stimulate or inhibit cytokine expression. Antiviral and antibiotic drugs may be used in part to test hypotheses concerning persistent viral or bacterial infection of oligodendrocytes or other glial supporting cells. Other potential treatments include interventions that reduce free oxygen species-induced tissue injury and metabolic cell injury cascades. Finally, administration of growth factors, tissue transplantation, and gene therapy may be used to target specific enzymatic, chemical, or mechanistic defects when identified in the future.

Most of the current therapies shown to alter the short-term or intermediate-term (1–4 years) course of MS have rather broad mechanisms of action. For instance, corticosteroids, which hasten recovery from exacerbations, modulate the activity of transcription factors such as activator protein-1 (AP-1) and nuclear factor κB (NF-κB), which in turn reduce the expression of proinflammatory genes (De Bosscher et al, 2000). This results in reduced T cell migration and inhibition of humoral effects (Cato and Wade, 1996). The beta-interferons, which reduce exacerbation frequency and may slow the accumulation of neurological disability for at least 2 to 4 years (longer studies have not yet been done), also have wide-ranging immunological effects (Yong et al, 1998a). These include effects on antigen presentation, inhibition of lymphocyte proliferation, shifting cytokine secretion towards an anti-inflammatory profile, and inhibition of MMPs. The modest clinical benefit that these therapies bestow, despite their breadth of immunological effects, suggests that successful future therapies will likely require multifaceted actions that may only be achieved by combination therapies targeted against both specific pathological patterns of MS and nonimmune pathophysiological processes yet to be discovered.

Conclusion

There has been significant advancement in elucidation of the mechanisms operative in MS pathogenesis. Many components of individual processes thought to be important in contributing to pathways ending in demyelination or axonal injury have been studied in detail. Many fundamental questions, however, still remain, including the following: Is MS one or a spectrum of diseases? What triggers the onset of the disease? What are the autoantigens? Does pathological heterogeneity mean that many environmental agents may incite the disease? Or does it mean that different gene combinations portend variable disease susceptibility and different pathologies? What causes axonal injury? What are the critical factors limiting remyelination?

In most instances, clues to these questions are limited to circumstantial, in vitro, or animal model evidence. The task of advancement is made more difficult by the clinical, radiological, and pathological heterogeneity of MS and the relative inaccessibility of human pathological samples. More sophisticated animal models, inferences from therapeutic clinical trials, and noninvasive brain imaging techniques are reducing these challenges. Further therapeutic progress for this disabling disease will likely require integration of several complementary interventions that target a variety of important steps in the pathogenic process.

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