Pathogenesis of myelin/oligodendrocyte damage in multiple sclerosis

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ABSTRACT Substantial evidence supports autoimmune activity as the etiologic mechanism underlying multiple sclerosis (MS). Both the innate and the adaptive arms of the immune system are involved in the aberrant response to several antigens associated with the myelin sheath and oligodendrocytes (OGCs) after the activation of immune cells by self- or cross-reactive microbial pathogens. The $CD4^+$ Th1 cell, in particular, has been implicated, but it is abetted by a variety of other cell types ($CD8^+$ cells, B cells, macrophages, and microglia) and soluble products (proteases, cytokines, and nitric oxide [NO]) that act both outside of and within the CNS. This review describes recent and salient findings from animal models and human clinical studies that have established our current understanding of the distinct steps in the development of immune autoreactivity that culminates in the CNS lesions associated with MS. **NEUROLOGY 2007;68 (Suppl 3):S13–S21**

The inflammatory cascade associated with multiple sclerosis (MS) involves many of the known effector components of the immune system that typically act in an interdependent and stepwise fashion. For example, the autoreactive Th1 cells that are integral to MS disease development must be activated, adhere to, and then migrate across the endothelium of the CNS, escape the regulatory functions of other cellular components of the immune system, and ultimately attack, either directly or indirectly, the oligodendrocytes (OGCs), myelin sheaths, and axons to form the lesions associated with disease. Figure 1 presents an overview of the immune system components involved in MS.[1] An understanding of this multistep process has made possible the development of interventional strategies aimed at several defined components of the immune system, and a number of these strategies are proving successful in reducing the rate of initial formation of CNS lesions. Once inflammation sets in within the CNS, local inflammatory conditions allow a secondary type of damage to develop, which in many cases continues to progress in spite of immunomodulatory therapy. This stage of the disease process might potentially be amenable to neuroprotective therapies. These therapies, of course, must take into consideration the molecular nature of the various types of immune insults that created the lesions in the first place. This article focuses on the clinical and experimental evidence that supports current understanding of the mechanisms of inflammation that lead to demyelination and OGC injury as a basis for further discussion of potential neuroprotective strategies.

PERIPHERAL T-CELL ACTIVATION

The targets of the autoimmune response in MS are believed to be cellular components of the CNS that are normally inaccessible to the immune system because of their location behind the blood–brain barrier (BBB). Entry of immune cells into the CNS depends on their state of activation and their ability to respond to cytokine and chemokine signals that induce them to cross the BBB. The inflammatory infiltrate in active MS lesions consists of a mostly perivascular accumulation of $CD4^+$ and $CD8^+$ T cells, monocytes, and B cells, and it eventually includes macrophages in the lesion center that stain positively for ingested myelin (for review see Hafler et al.[2]).

Activation of immune cells outside of the CNS (e.g., in the peripheral lymph nodes) results from functional recognition of antigenic peptides by T-cell receptors (TCRs) on the $CD4^+$ lymphocyte surface that are presented in the context of major histocompatibility complex (MHC) class II mole-

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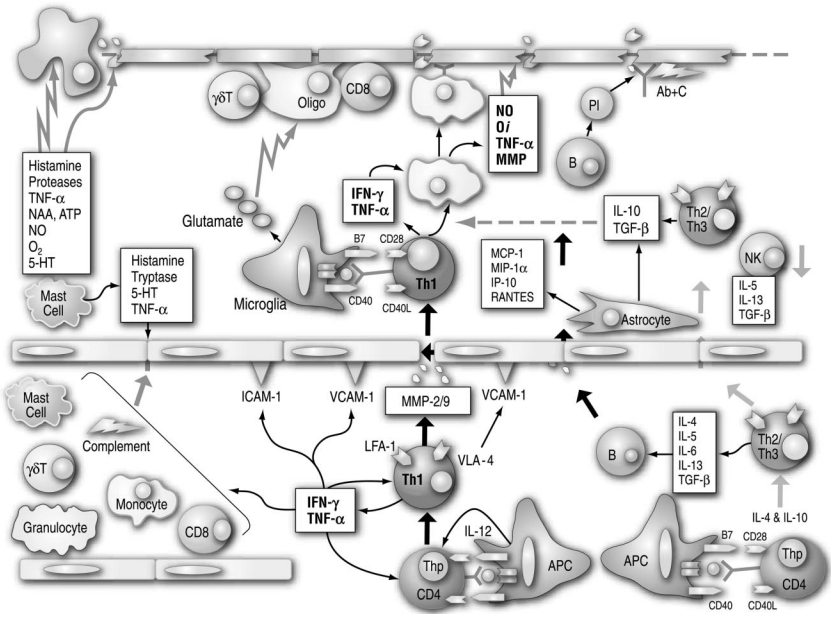
Figure 1 Overview of the components of the immune system that are involved in pathogenesis in MS, as discussed in the text



Ab + C, antibody plus complement; APC, antigen-presenting cell; ATP, adenosine triphosphate; B, B cell; 5-HT, 5-hydroxytryptamine; ICAM-1, intercellular adhesion molecule 1; IFN, interferon; IL, *interleukin; IP-10, interferon γ-inducible protein-10; LFA-1, lymphocyte function-associated antigen 1; MMP, matrix metalloproteinase; MS, multiple sclerosis; NAA, N-acetyl aspartate; NO, nitric oxide; O_i, free oxygen radicals;PI, plasma cell; RANTES, Regulated on Activation, Normal T–cell Expressed and Secreted; TGF-β, transforming growth factor β; Th, T helper; Thp, T-helper precursor; TNF-α, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule 1; VLA-4, very late antigen 4. From Dhib-Jalbut et al.,[1] with permission.*

cules and accessory molecules by antigen-presenting cells (APCs). The nature of the autoantigen that triggers MS is not precisely known, but antigenic peptides relevant to MS and experimental autoimmune encephalomyelitis (EAE) may be derived from a number of myelin-associated proteins, including proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), and myelin basic protein (MBP). Myelin-reactive T cells are found in the blood, cerebrospinal fluid (CSF), and brain tissue of MS patients.

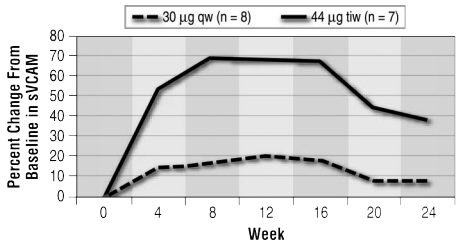
Particular amino acid sequences of MBP have proved to be immunodominant, and blood from MS patients frequently contains T-cell clones specific for a peptide comprising amino acids 85 to 99 (p85–99). In humans bearing the human leukocyte antigen (HLA) DRB1*0401 allele, amino acids 111 to 129 (p111–129) from MBP are immunodominant. The relevance of this epitope to MS pathology was demonstrated by Quandt et al.[3] using a model in which mice made transgenic for the genes encoding the DRB1*0401 allele were infused with T cells from mice made transgenic for the TCR expressed by the human $CD4^+$ T-cell line MS2–3C8 specific for amino acids p111–129. The recipient mice developed hindlimb ascending paralysis and had cellular infiltrates in the brain, spinal cord, and spinal nerve roots that included the transgenic T cells. The oligoclonality and specificity of autoreactive T cells in MS suggest that myelin-associated proteins are the predominant activating antigens. Features of the cellular immune system, including how such cross-reactive T cells are allowed to exist in the first place, have revealed a much more complex story.

The thymic selection process, by which T cells that strongly recognize self-antigens are removed, allows the survival of cells that weakly bind such antigens. Studies performed nearly 10 years ago showed that the essential attributes of peptides that bind to TCRs are more related to space-filling properties of side-chain structure than to specific amino acid sequence. By systematically modifying the amino acids of an 11-mer from MBP p86–96 and testing the ability of the peptides to activate a p86–96–specific $CD4^+$ T-cell clone, investigators identified several members of the resulting peptide libraries that activated the T cells, in some cases at much lower concentrations than the original peptide.[4] This information about the essential structural requirements of this TCR was used to successfully search protein databases for peptides from other self- and microbial proteins that were capable of activating the same T-cell clone. A slightly different experimental approach to identify antigenic microbial peptides yielded 129 peptides from a protein database that fit the structural requirements to bind to a series of MBP-specific T-cell clones from patients with MS. When tested with seven such clones, one bacterial and seven viral peptides were found that activated three of the clones. The viral sources of the peptides included *Herpes simplex*, adenovirus type 12, human papilloma virus, Epstein-Barr virus, and influenza A; the bacterial peptide was from *Pseudomonas aeruginosa*.[5] In general, therefore, for some autoreactive $CD4^+$ T-cell clones, antigen recognition, i.e., TCR specificity, is degenerate, and the self-antigen that induces proliferation of a T-cell clone is not the only ligand to which that clone can bind. This, of course, permits the consideration that autoimmune diseases, including MS, may be triggered by immune responses to relatively common infectious agents that express epitopes that cross-react with self-antigens through a process of molecular mimicry.

Evidence that autoreactive T cells play a role in MS relapses and disease activation has come from clinical trials designed to exploit the degeneracy of the TCR. Building on results of animal models in which immunotherapy of T-cell–mediated autoimmune disease was achieved using altered versions of immunogenic peptides, an altered peptide ligand (APL) was made (CGP77116) based on MBP p83–99. This was administered to 24 patients with relapsing–remitting MS (RRMS) to test its ability to act either as a partial agonist, a TCR antagonist, or an inducer of T-cell bystander suppression.[6] In this phase II trial, APL was found to exacerbate MS

From Graber et al.,[12] with permission.

Figure 2 Levels of soluble vascular cell adhesion molecule (sVCAM) during IFN-β1a treatment ($p < 0.001$)



symptoms in three patients, and an increased and heterogeneous response of $CD4^+$ T cells specific for MBP p83–99 was found. A portion of the CGP77116 was sufficiently similar to the actual MBP peptide to stimulate HLA-restricted $CD4^+$ T cells specific for the native form of the peptide. The dose of the administered APL appeared to influence the direction of the response because high concentrations induced Th1 (proinflammatory) responses, whereas a low dose induced Th0 or Th2 (anti-inflammatory) responses. Therefore, MBP-specific $CD4^+$ T cells are directly involved in MS relapse and disease activation, and a single APL will not limit the activity of all self-reactive T-cell clones. Further support for the use of APL as immunomodulators was provided in another phase II trial of a multiply substituted MBP p83–99 peptide.[7] In this case, low doses of peptide were secondarily associated with a reduced number and volume of gadolinium (Gd)-enhancing lesions and with a cytokine profile indicative of a Th2-type response.

Direct evidence that an infectious agent could trigger an autoimmune condition as a result of the degeneracy of TCR ligand specificity was provided by Olson et al.[8] using an encephalitogenic virus. In a series of experiments with this model, Theiler's murine encephalomyelitis virus was engineered to express either a peptide from murine myelin PLP139–151 or a mimetic peptide expressed by *Haemophilus influenzae*. Infection of mice with the recombinant viruses induced early-onset paralytic disease, which was associated with activation of $CD4^+$ T cells that could cross-react with native. The finding of T cells with highly degenerate ligand specificities that could cause disease when activated implied the presence of a mechanism that normally regulates the activity of these cells. It was also suggested that the site of infection by the microbe may play a role in modifying the environment in which disease develops, but this could not be tested with the encephalotrophic virus used in this system.

T-CELL ADHESION: FROM THE PERIPHERY TO THE BBB After their activation in the periphery, autoreactive T cells and activated monocytes must access and bind to the BBB. Extensive animal experimental data demonstrate the role of cognate pairs of adhesion molecules on T cells and endothelial cells that are essential in this process. EAE models have shown that when $CD4^+$ T-cell clones specific for MBP are injected IP, they are found in the CNS after 4 to 12 hours, with hindlimb ascending paralysis developing 4 to 5 days after injection. Yednock et al.[9] showed that binding of these lymphocytes and macrophages to brain tissue sections was dependent on $\alpha 4\beta 1$ integrin. Binding of cells to the tissue sections was inhibited by anti-$\alpha 4$ antibody, and the same antibody prevented the accumulation of lymphocytes in the CNS and the development of EAE. Two lymphocyte adhesion molecules and their binding partners have been implicated in MS. The β-integrin very late antigen-4 (VLA-4) and lymphocyte function-associated antigen (LFA-1), and their vascular and intercellular cell adhesion molecules, vascular cell adhesion molecule 1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1), respectively, are all upregulated in vessels associated with MS lesions. Interestingly, there is greater upregulation of VLA-4 and VCAM-1 in vessels of chronic lesions than in vessels of acute lesions (whereas LFA-1 and ICAM-1 were high in both), suggesting a possible switch to their preferential use in late stages of lesion development.[10] The ability of interferon (IFN)-β to reduce the number of relapses, the rate of MS progression, and the number of Gd-enhancing lesions suggests that its mode of action includes regulating the access of immune cells to the BBB. Muraro et al.[11] measured the levels of VLA-4 and LFA-1 on T cells of MS patients 2 months before and for 3 months into IFN-β treatment. MRI findings correlated with a decreased level of VLA-4 on $CD8^+$ cells and $CD4^+/CD45RO^+$ memory cells, again suggesting that IFN-β affects the manner in which T cells interact with the BBB. These studies focused on cell membrane–bound versions of the vascular cell adhesion molecules (mVCAMs), but soluble forms of VCAM (sVCAMs) in blood exist in levels that could affect the ability of T cells to adhere to endothelium. Although the precise mechanism is unclear, studies with both IM and SC IFN-β1a formulations in patients with RRMS resulted in increased levels of sVCAMs in both treated groups relative to pre-IFN treatment levels (figure 2). A 14% increase in sVCAM levels was seen in the IM IFN-β1a study, in which patients received 30 μg once weekly, compared with a 56% increase in the SC IFN-β1a study,

in which patients received 44 μg three times weekly.[12] The inversely correlating reductions in MRI lesions in the treatment groups suggest that increased amounts of sVCAM may serve to bind to the VLA-4 on circulating T cells and thereby prevent their interaction with mVCAMs on CNS endothelium. The most direct demonstration of the role of VLA-4 in the adhesion step of the inflammatory cascade comes from a study using the monoclonal antibody natalizumab, which binds to VLA-4.[13] During this 2-year phase III trial in patients with RRMS, the rate of relapse in the treated cohort group was reduced by 68% at 1 year, Expanded Disability Status Scale (EDSS) score was reduced by 42% at the end of year 2, and there were 82% fewer T2-weighted MRI new or enlarging hyperintense lesions and 92% fewer Gd-enhancing lesions. As would be expected of an intervention that inhibited access of T cells to the CNS, the effects of natalizumab treatment were apparent early and lasted throughout the study period. These results may call into question the notion that MS begins as a degenerative process within the CNS for, if true, the subsequent blocking of entry of T cells would not be expected to have an effect on lesion progression.

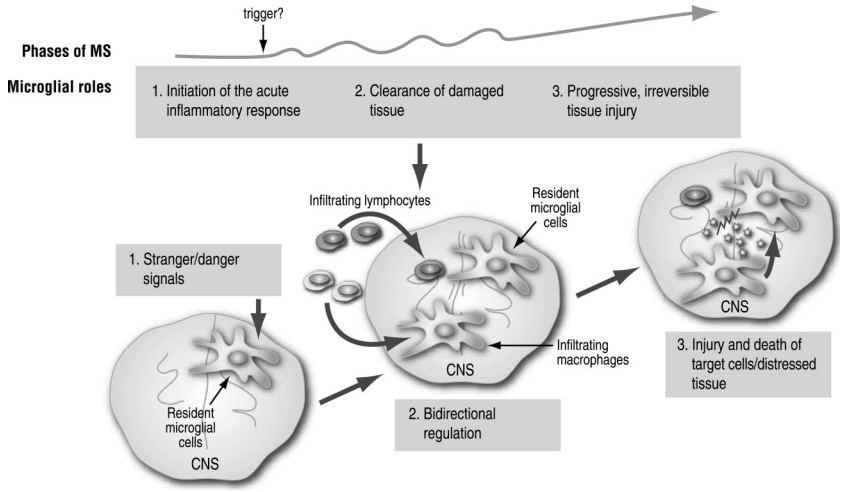
T-CELL MIGRATION INTO THE CNS The next step in the inflammation process is the crossing of the BBB by activated immune cells. Degradation of the basement membrane underlying endothelial cells may result from overactivity of matrix metalloproteinases (MMPs), which are produced by a variety of cell types, including monocytes, macrophages, T cells, and endothelial cells. They are also made by cells of the CNS, such as microglia, astrocytes, and oligodendrocytes, and are used for remodeling and repair of cell matrix components. The activity of these enzymes is normally regulated by tissue inhibitors of MMP (TIMPs). Increased levels of the MMP gelatinase B have been associated with EAE and have been correlated with increased damage to and crossing of the BBB by immune cells. Several studies have demonstrated a role for MMPs and TIMPs in MS (table 1). In a prospective study of gelatinase B, TIMP-1, and TIMP-2 in patients with RRMS, levels of all three proteins were found to be elevated in the patients compared with healthy controls. Gelatinase B levels were significantly higher during clinical relapses than during clinically quiescent periods, whereas levels of TIMP-1 were unchanged and those of TIMP-2 decreased slightly.[14] This pattern of TIMP expression in MS was different from that in unrelated CNS inflammatory conditions. Changes in the relative levels of a different MMP and its inhibitory TIMP were found in another study of patients with RRMS.[15] In this case, serum MMP-9 levels were elevated in the patients compared with healthy controls, but TIMP-1 levels were not. This change in the ratio was found to precede the appearance of new Gd-enhancing lesions and was detectable in serum during the month before lesion development. The authors therefore proposed that events occurring in the periphery, not in the CNS, result in altered ratios of MMP/TIMP that affect initial stages of MS. Similar results in terms of ratio changes were reported in a more recent study using IFN-β in which serum MMP-9 and TIMP-1 levels in patients with RRMS were followed.[18] In this case, IFN-β1a treatment caused no change in MMP-9 levels but did cause an increase in TIMP-1 levels at the 3- and 6-month test points. This effect was no longer apparent at 12 months. Further evidence supporting a role for MMPs comes from a different IFN-β study. Trojano et al.[16] reported that in a 2-year study of RRMS patients receiving INF-β1b, as concentrations of soluble ICAM increased and levels of MMP-9 decreased during the first year there was a parallel clinical improvement (in terms of relapse frequency, EDSS score, and Gd-enhancing MRI lesions). Levels of soluble ICAM and TIMP-9 returned to baseline during the second year, and this was accompanied by a partial loss in clinical improvement.[16] Lastly, inhibition of MMP activity in mice also supports a role for this class of proteinases in mediating the crossing of the BBB by leukocytes.[19] In this study, the metal-chelating antibiotic minocycline was shown to inhibit MMP-9 activity, decrease production of MMP-9 protein, decrease the migration of T cells through fibronectin matrix barriers, reduce the infiltration of leukocytes into the CNS, and reduce EAE disease severity.

Table 1 *Evidence for MMP involvement in MS*

There is good correspondence between elevated serum MMP levels and Gd-enhanced MRI[14,15]
Serum MMP-9 content is decreased in patients receiving IFN-β[16]
The number of MMP-9–expressing leukocytes is reduced in patients receiving IFN-β[17]
IFN-β treatment increases serum TIMP-1[18]

INSIDE THE CNS: THE OLIGODENDROCYTE/MYELIN ATTACK Once within the CNS, Th1 cells must be stimulated to be retained there, and the resident microglial cells are believed to be responsible for this. These cells have been implicated in all stages of MS disease development, including serving as antigen-presenting cells to activate the cells that cause CNS tissue damage, clearing away damaged tissue and cells after relapses, and contributing to

Figure 3 Role of microglia in the development of MS lesions, as discussed in the text



From Jack et al.,[20] *with permission.*

the irreversible damage associated with the chronic phase (figure 3). As members of the innate arm of the immune system, microglia express Toll-like receptors which, on binding to pattern-type microbial antigens, activate the cells to secrete the proinflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6. Microglia may also be induced to express MHC class II molecules with which to present antigen to cells of the adaptive arm of the immune system. They can be activated in response to cues from the periphery and, in turn, influence the activity and function of infiltrating T cells and macrophages. Activated T cells may cause damage within the CNS, both directly and indirectly, whereas activated microglia and macrophages themselves cause damage through release of toxic compounds that induce apoptosis and by ingestion of apoptotic OGCs (for review see Jack et al.[20] and Antel[21]).

Evidence for the activation of microglial cells in MS includes the demonstration of ligand binding to peripheral benzodiazepine receptors in active MS lesions. Binding of both PK11195 (to peripheral benzodiazepine receptors) and MAC1/CD11b (a marker of activated macrophages) was increased in the edges of MRI-defined active MS lesions.[22] Evidence suggesting that microglial activation may be an early event in lesion development was also revealed in a pathologic analysis of brain tissue samples from patients with RRMS who died during or just after onset of a relapse. Barnett and Prineas[23] reported finding changes to cellular architecture that occurred in the absence of leukocyte infiltration, including apoptosis of OGCs and activation of microglia. This finding raises the possibility that microglial cells initiate lesion development before activation in the periphery has taken place.

The consequences of microglial activation have thus far been best demonstrated in animal models. Through expression of Toll-like receptor-4, microglia may be activated on binding of lipopolysaccharide (LPS). In vitro, mixed cell cultures of rat forebrain treated with LPS suffered loss of axons, OGCs, and microglia.[24] In similar cultures devoid of microglia, there was no loss of axons on addition of LPS. More specifically, Toll-like receptor 4-deficient mice, which are unresponsive to LPS, do not suffer axon loss on combined LPS/hypoxia–ischemia treatment, whereas similarly treated normal mice do. These results serve to link innate immunity and neuron toxicity and also show that irreversible CNS damage may result from two types of insult: an initial potentially reversible one (such as hypoxia–ischemia) and a second one that takes advantage of the compromised state of the cells that were exposed to the first to induce irreversible damage. The microglial products responsible for neuronal toxicity were not defined in this study but might include IL-1β, IL-6, TNF-α, and/or NO, all of which are known to be neurotoxic in vitro. Activated microglia may also have an impact on the regeneration of neurons. Neurogenesis experimentally induced in mice by brain insult is impaired by concomitant administration of LPS, perhaps indicating a particular sensitivity of new neurons to products released by activated microglia.[25] A direct demonstration of the expression of products that could serve such a function was made by Mycko et al.,[26] who used microarrays to analyze gene expression in the cells found in both the lesion margins and centers of chronic active and inactive MS lesions. Chronic active lesions were found to express more unique genes in their margins than inactive lesions. Of particular relevance to potential neurotoxicity mechanisms, transcripts from genes encoding TNF-α and IL-6 were found in cells in both the margins and centers of active lesions but not of inactive lesions. As an example of adding injury to insult, functionally compromised neurons, as may result from interaction with products of activated microglia, are associated with excessive levels of extracellular glutamate, which is toxic to OGCs. Riluzole inhibits release of glutamate from nerve terminals and modulates kainate and *N*-methyl-D-aspartate receptors. Patients treated with riluzole for primary progressive MS have significantly reduced rates of development of T1-hypointense MRI lesions but only slightly decreased rates of brain atrophy. This suggests that extracellular glutamate has an effect on MS lesion progression and axon loss but not on new lesion formation.[27]

Another product of activated microglia that may contribute to MS pathogenesis is NO. Many cell

types express inducible NO synthase (iNOS), including activated macrophages and microglia, and iNOS expression is abundant in the edges of MS lesions.[28] Generated NO may combine with superoxide to form peroxynitrite, which nitrosylates certain amine and sulfate side groups on proteins and lipids. Nitrolysine and fragments of MBP are recognized as markers of myelin damage, and both are found in MS plaques. Others have described upregulated levels of iNOS mRNA and protein in the CNS of EAE models and in lesions of patients with MS.[29] Although these results indicate a role for NO in MS pathology, the therapeutic regulation of iNOS expression in MS is not expected to be straightforward. NO is involved in normal immune functions, and inhibitors of iNOS may therefore have both beneficial and deleterious effects.

After infiltration and reactivation by microglia, Th1 cells may cause both indirect and direct CNS damage. As an example of an indirect effect, $CD4^+$ T-helper cells secrete a number of proinflammatory products, including IL-2, an autocrine stimulator of cell division, and IFN-γ, which can activate macrophages and microglia, as well as $\gamma\delta$ T cells, to kill other cell types. IFN-γ also induces Th0 cells to differentiate into a Th1 phenotype. Further amplification of this effect of Th1 products follows IL-12 release from macrophages that induce Th1 cells to secrete more IFN-γ (for review, see Parkin and Cohen[30]).

Direct damage to OGCs and the myelin sheath results from a number of cell products and cell types. Fc receptors (FcRs) connect the humoral and cellular divisions of the immune system by allowing controlled interactions between antibodies and effector cells. Type I FcRs, found on macrophages and neutrophils, and type III FcRs, found on the same cells and on mast cells, $\gamma\delta$ cells, and NK cells, promote phagocytosis and antibody-dependent, cell-mediated cytotoxic activation of inflammatory cells. Type II FcRs, on the other hand, found on B cells and myeloid phagocytic cells, downregulate immune responses. An analysis of FcR knockout mice induced to develop EAE with MOG showed that lack of FcR II served to enhance EAE disease by allowing increased demyelination. FcR I and III knockouts, however, developed a peripheral response to MOG but did not show signs of CNS destruction.[31] FcRs therefore appear to be involved in the secondary immune response in the CNS, at least in the EAE model.

A distinct cell type, the mast cell, has also been implicated in direct toxic effects within the CNS and has been shown to have a spatial association with MS plaques. Mast cells express FcR I and can release their contents in an IgE-dependent or IgE-independent manner. Released products include several that have effects on the BBB and immune cells within the CNS. For example, the vasoactive agents histamine, TNF-α, and tryptase may alter blood vessel permeability, and IL-16 and histamine act as chemoattractants for leukocytes and induce them to attach and roll along the endothelium before extravasation. Mast cells may influence the direction of T-helper lineage development and may also directly degrade myelin by secretion of proteases (for review, see Zappulla et al.[32]). Like NO, however, the essential and widespread role of mast cells will probably make therapeutic targeting of these cells in MS difficult.

Activation of $CD4^+$ Th1 cells is important as an initial step in autoimmune responses, but $CD8^+$ and $\gamma\delta$ cells, along with antibody and complement, may also mediate myelin attack. $CD8^+$ cells actually outnumber $CD4^+$ cells in MS lesions, and they can recognize antigens in the context of MHC class I, which can be upregulated on the surface of damaged neurons. Class I-restricted epitopes have been defined within the same MBP, PLP, and MOG proteins that activate class II-restricted $CD4^+$ cells, and cytotoxic $CD8^+$ cells have a direct ability to lyse targets. Although much less is known about $\gamma\delta$ T cells in MS, they can directly lyse OGCs via release of perforin, and a distinct subtype of $\gamma\delta$ T cell with limited antigen specificity is found in CSF infiltrates from patients with MS and in EAE models (for review, see Sospreda and Martin[33]). B cells and their antibody products also contribute to lesion development. Although controversial, antibodies that recognize MOG in particular are increased in the CNS of patients with MS, and the presence of such antibodies may be predictive of definitive MS diagnosis and future exacerbations.[34] Anti-MOG antibodies have been shown to cause destruction of myelin in EAE models, but antibodies to MBP or PLP have not.[2] The presence of complement-secreting cells in the CNS also allows the active components of this system to participate in MS lesion development. Autoimmune responses against myelin allow activation of the classic complement pathway, resulting in lysis of OGCs and chemoattraction of macrophages and their subsequent phagocytosis of antibody- and complement-opsonized OGC fragments.

Also of growing interest in MS lesion development is the role of chemokines, the small proteins that direct movement of circulating leukocytes to sites of inflammation. In the CNS, both astrocytes and Th1 cells are known producers of chemokines, and the great majority of cells in MS lesions are infiltrated $CD4^+$ cells, $CD8^+$ cells, and macrophages,

Table 2 *Regulatory cells in MS*

Cell type	Mediators
Tr1	IL-10
Th2	IL-4, IL-13, IL-5
$CD4^+/CD25^+$	Uncertain
Th3	TGF-β
$CD8^+$	Cytolytic
Astrocytes	IL-10, TGF-β

IL = interleukin; TGF-β = transforming growth factor β.

underscoring the important role of active recruitment and retention of immune cells by these compounds. Of the 10 families of chemokine receptors, CCR1 and CCR2 have definite connections with MS. Patients with MS have reduced levels of the chemokine CCL2 in CSF compared with normal subjects or those with noninflammatory disorders, which may result from its consumption by the significant number of CCR2-bearing infiltrating cells. Phagocytic macrophages, such as are found in MS lesions, express CCR5, which binds a wide array of chemokines found in MS lesions. These and other data showing changes in the profile of chemokine receptor expression by T cells and macrophages in MS lesions suggest that these compounds play significant and complex roles in the establishment and maturation of MS lesions.[35]

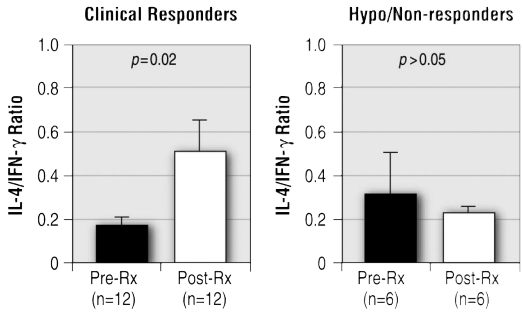
REGULATION OF INFLAMMATORY RESPONSE AND RECOVERY There is also increasing evidence of active regulation that leads to recovery from the autoimmune activity in MS lesions, based on EAE models and human studies. A summary of these regulatory cells is provided in table 2. In the EAE model, Th2 cells generated within the CNS have been shown to produce regulatory molecules IL-4, IL-10, and TGF-β. Cells with regulatory function in humans with MS have been demonstrated by Hafler's group.[36] The equivalent of $CD4^+/CD25^+$ suppressor T cells in mice are $CD4^+/CD25^{hi}$ cells in humans, which suppress the activation, proliferation, and effector functions of activated responder T cells. These investigators reasoned that because autoreactive T cells in patients with MS are more easily activated than those in healthy subjects, there might be a deficiency in the production or function of $CD4^+/CD25^{hi}$ cells. Comparing RRMS patients with healthy controls, they found that $CD4^+/CD25^{hi}$ cells were equally numerous in the two groups, with 10% of the $CD4^+$ cells also expressing CD25 and 1% to 2% expressing $CD25^{hi}$ levels. However, there was a diminished function of this cell population in patients with MS. Specifically, $CD4^+/CD25^{hi}$ cells from normal subjects efficiently suppressed proliferation and IFN-γ production of responder cells when combined in a 1:1 ratio, but the same cell types from patients with MS did not suppress either response. Mixing experiments showed that this was not due to increased resistance of responder cells or to an increased number of activated responder cells in MS patients. Rather, there was a smaller fraction of the $CD4^+/CD25^{hi}$ population in MS patients (17%) that had regulatory activity than there was in normal controls (60%), revealing a defect in this regulatory cell population in MS patients.

Further evidence for a role of regulatory cells derives from studies with glatiramer acetate (GA), in response to which Th2 and Th3 cells are generated and enter the CNS. To follow the influx of such cells into the brain and to monitor their production of anti-inflammatory cytokines, Aharoni et al.[37] dye-labeled GA-specific T cells and transferred them into normal or EAE mice. Sites of GA-specific cells in the brain also demonstrated increased levels of secreted brain-derived neurotrophic factor (BDNF), which is known to induce axon outgrowth, myelination, regeneration, and rescue damaged neurons. Also upregulated at these sites were two anti-inflammatory cytokines, IL-10 and TGF-β. These last two products were also being made by resident astrocytes and microglia. The GA-specific Th2/3 cells therefore have the ability to reduce inflammation and stimulate regeneration and repair of the lesion sites in the EAE model. GA-reactive T cells isolated from GA-treated patients with MS are Th2-biased and secrete BDNF.[38–40] The observation that GA treatment reduces the number of MRI Gd-enhancing lesions that progress into persistent black holes is consistent with a central mechanism of action for GA in humans, possibly mediated by Th2 cytokines and BDNF release.[41] Furthermore, regulatory T cells expressing foxp3 have been described in response to GA,[42] suggesting a role for these cells in GA's mechanism of action and supporting the importance of regulatory T cells in modulating the inflammatory response in MS.

In a study of patients with RRMS, GA-reactive T cells were analyzed for cytokine receptor profile and cytokine production during 12 months of treatment.[43] The patients demonstrated reduction in the expression of cytokine receptors CXCR3, CCR5, and CXCR6 on GA- and myelin-reactive T cells. The reduction of CXCR6 was significant because this receptor is expressed on Th1 cells in the periphery, indicating bystander modulation of cytokine receptors in the periphery as well as in the CNS. In addition, after 1 year of treatment there was an increase in the expression of CCR7, which increases

This suggests that GA-induced anti-inflammatory cytokine shifts can function to modulate the clinical course of MS. GA, glatiramer acetate; IFN-γ, interferon γ; MS, multiple sclerosis; Rx, drug treatment.

Figure 4 The GA-induced IL-4:IFN-γ ratio was greater in responders than in nonresponders



homing to lymph nodes. GA therefore appeared to be causing a bystander effect in lymph nodes, thereby reducing the number of myelin-reactive effector cells entering the CNS. The timing of increased expression of CCR7 is also consistent with time-dependent changes from a Th1- to a Th2-type immunologic response. Finally, to determine whether a correlation exists between the GA-induced up-regulation of Th2-type cytokine profile and clinical presentation, patients with RMSS who had been receiving GA for 2 years were examined for their ratio of IL-4 (Th2) to INF-γ (Th1).[44] After 2 years, patients were divided into groups of responders (<0.5 increase in annual relapse rate) and nonresponders (relapse rate >1 and EDSS score progression ≥1 unit). The GA-induced Th2 shift, as revealed by the cytokine ratio, was greater in responders than in nonresponders, supporting a role for GA-induced Th2 cells in modulating the clinical course of MS (figure 4).

CONCLUSIONS The precise trigger for the body's autoimmune attack on components of myelin that leads to MS is unknown but may result from infection and/or genetically determined immune system responses or from aberrant microglial activation and oligodendrocyte apoptosis. As these alternatives imply, the anatomic site of the initiating event is also not clear at this time. Regardless of the autoimmune trigger, most patients with MS experience repeated attacks on OGCs and myelin via an inflammatory cascade that is activated repeatedly over time. The duration of these inflammatory events in MS is unknown, but they appear to constitute an early phase in development of the MS lesion. Eventually, the body's recovery mechanisms can no longer repair injured OGCs and depleted myelin, and irreversible neuron damage develops. An increasingly sophisticated knowledge of the immune pathways involved in early and late phases of MS has allowed a detailed understanding of the pathogenetic mechanisms underlying the disease. Surprisingly, treatments that target individual components of the immune response are proving effective at reducing MS disease progression, thereby revealing the interdependence of these components. This suggests that combinations of therapies, some designed to reduce the inflammation-driving aspects of the immune system and some to promote neuron protection and regeneration, might be highly effective in managing MS.

REFERENCES

1. Dhib-Jalbut S, Arnold DL, Cleveland DW, et al. Neurodegeneration and neuroprotection in multiple sclerosis and other neurodegenerative diseases. J Neuroimmunol 2006;176:198–215.
2. Hafler DA, Slavik JM, Anderson DE, O'Connor KC, De-Jager P, Baecher-Allan C. Multiple sclerosis. Immunol Rev 2005;204:208–231.
3. Quandt JA, Baig M, Yao K, et al. Unique clinical and pathological features in HLA-DRB1*0401–restricted MBP 111–129–specific humanized TCR transgenic mice. J Exp Med 2004;200:223–234.
4. Hemmer B, Fleckenstein BT, Vergelli M, et al. Identification of high potency microbial and self ligands for a human autoreactive class II-restricted T cell clone. J Exp Med 1997;185:1651–1659.
5. Wucherpfennig KW, Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell 1995;80:695–705.
6. Bielekova B, Goodwin B, Richert N, et al. Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. Nature Med 2000;6:1167–1175.
7. Kappos L, Comi G, Panitch H, et al., and The Altered Peptide Ligand in Relapsing MS Study Group. Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. Nature Med 2000;6:1176–1182.
8. Olson JK, Ludovic Croxford J, Miller SD. Innate and adaptive immune requirements for induction of autoimmune demyelinating disease by molecular mimicry. Mol Immunol 2004;40:1103–1108.
9. Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N. Prevention of experimental autoimmune encephalomyelitis by antibodies against α4β1 integrin. Nature 1992;356:63–66.
10. Cannella B, Raine CS. The adhesion molecule and cytokine profile of multiple sclerosis lesions. Ann Neurol 1995;37:424–435.
11. Muraro PA, Leist T, Bielekova B, McFarland HF. VLA-4/CD49d downregulated on primed T lymphocytes during interferon-β therapy in multiple sclerosis. J Neuroimmunol 2000;111:186–194.
12. Graber J, Zhan M, Ford D, et al. Interferon β-1a induces increases in vascular cell adhesion molecule: implications for its mode of action in multiple sclerosis. J Neuroimmunol 2005;161:169–176.

13. Polman CH, O'Connor PW, Havrdova E, et al., for the AFFIRM Investigators. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 2006;354:899–910.
14. Lee MA, Palace J, Stabler G, Ford J, Gearing A, Miller K. Serum gelatinase B, TIMP-1 and TIMP-2 levels in multiple sclerosis: a longitudinal clinical and MRI study. Brain 1999;122:191–197.
15. Waubant E, Goodkin DE, Gee L, et al. Serum MMP-9 and TIMP-1 levels are related to MRI activity in relapsing multiple sclerosis. Neurology 1999;53:1397–1401.
16. Trojano M, Avolio C, Liuzzi GM, et al. Changes in serum sICAM-1 and MMP-9 induced by rINFβ-1b treatment in relapsing-remitting MS. Neurology 1999;53:1402–1408.
17. Özenci V, Kouwenhoven M, Teleshova N, Pashenkov M, Fredrikson S, Link H. Multiple sclerosis: pro- and anti-inflammatory cytokines and metalloproteinases are affected differentially by treatment with IFN-β. J Neuroimmunol 2000;108:236–243.
18. Karabudak R, Kurne A, Guc D, Sengelen M, Canpinar H, Kansu E. Effect of interferon β-1a on serum matrix metalloproteinase–9 (MMP-9) and tissue inhibitor of matrix metalloproteinase (TIMP-1) in relapsing remitting multiple sclerosis patients. J Neurol 2004;251:279–283.
19. Brundula V, Rewcastle NB, Metz LM, Bernard CC, Yong VW. Targeting leukocyte MMPs and transmigration: minocycline as a potential therapy for multiple sclerosis. Brain 2002;125:1297–1308.
20. Jack C, Ruffini F, Bar-Or A, Antel JP. Microglia and multiple sclerosis. J Neurosci Res 2005;81:363–373.
21. Antel J. Oligodendrocyte/myelin injury and repair as a function of the central nervous system environment. Clin Neurol Neurosurg 2006;108:245–249.
22. Vowinckel E, Reutens D, Becher B, et al. PK11195 binding to the peripheral benzodiazepine receptor as a marker of microglia activation in multiple sclerosis and experimental autoimmune encephalomyelitis. J Neurosci Res 1997; 50:345–353.
23. Barnnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol 2004;55:458–468.
24. Lehnardt S, Massillon L, Follett P, et al. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. Proc Natl Acad Sci USA 2003;100:8514–8519.
25. Ekdahl CT, Claasen J-H, Bonde S, Kokaia Z, Lindvall O. Inflammation is detrimental for neurogenesis in adult brain. Proc Natl Acad Sci USA 2003;100:13632–13637.
26. Mycko MP, Papoian R, Boschert U, Raine CS, Selmaj KW. Microarray gene expression profiling of chronic active and inactive lesions in multiple sclerosis. Clin Neurol Neurosurg 2004;106:223–229.
27. Killestein J, Kalkers N, Polman CH. Glutamate inhibition in MS: the neuroprotective properties of riluzole. J Neurol Sci 2005;233:113–115.
28. Hill KE, Zollinger LV, Watt HE, Carlson NG, Rose JW. Inducible nitric oxide synthase in chronic active multiple sclerosis plaques: distribution, cellular expression and association with myelin damage. J Neuroimmunol 2004; 151:171–179.
29. Encinas JM, Manganas L, Enikolopov G. Nitric oxide and multiple sclerosis. Curr Neurol Neurosci Rep 2005;5: 232–238.
30. Parkin J, Cohen B. An overview of the immune system. Lancet 2001;357:1777–1789.
31. Abdul-Majid K-B, Stefferl A, Bourquin C, et al. Fc receptors are critical for autoimmune inflammatory damage to the central nervous system in experimental autoimmune encephalomyelitis. Scand J Immunol 2002;55:70–81.
32. Zappulla JP, Arock M, Mars LT, Liblau RS. Mast cells: new targets for multiple sclerosis therapy? J Neuroimmunol 2002;131:5–20.
33. Sospedra M, Martin R. Immunology of multiple sclerosis. Annu Rev Immunol 2005;23:683–747.
34. Berger T, Rubner P, Schautzer F, et al. Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. N Engl J Med 2003; 349:139–145.
35. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med 2006;354:610–621.
36. Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by $CD4^+CD25^+$ regulatory T cells in patients with multiple sclerosis. J Exp Med 2004;199:971–979.
37. Aharoni R, Kayhan B, Eilam R, Sela M, Arnon R. Glatiramer acetate-specific T cells in the brain express T helper 2/3 cytokines and brain-derived neurotrophic factor *in situ*. Proc Natl Acad Sci USA 2003;100:14157–14162.
38. Chen M, Valenzuela RM, Dhib-Jalbut S. Glatiramer acetate-reactive T cells produce brain-derived neurotrophic factor. J Neurol Sci 2003;215:37–44.
39. Dhib-Jalbut S, Chen M, Said A, Zhan M, Johnson KP, Martin R. Glatiramer acetate-reactive blood mononuclear cells respond to multiple myelin antigens with a Th2-biased phenotype. J Neuroimmunol 2003;140:163–171.
40. Farina C, Weber MS, Meinl E, Wekerle H, Hohlfeld R. Glatiramer acetate in multiple sclerosis: update on potential mechanisms of action. Lancet Neurol 2005;4:567–575.
41. Comi G, Filippi M, Wolinsky JS, for the European/Canadian Glatiramer Acetate Study Group. European/Canadian multicenter, double-blind, randomized, placebo-controlled study of the effects of glatiramer acetate on magnetic resonance imaging–measured disease activity and burden in patients with relapsing multiple sclerosis. Ann Neurol 2001;49;290–297.
42. Hong J, Li N, Zhang X, Zheng B, Zhang JZ. Induction of $CD4^+CD25^+$ regulatory T cells by copolymer-I through activation of transcription factor Foxp3. Proc Natl Acad Sci USA 2005;102:6449–6454.
43. Allie R, Hu L, Mullen KM, Dhib-Jalbut S, Calabresi PA. Bystander modulation of chemokine receptor expression on peripheral blood T lymphocytes mediated by glatiramer therapy. Arch Neurol 2005;62:889–894.
44. Valenzuela RM, Costello K, Johnson KP, Dhib-Jalbut S. Ex-vivo cellular IL-4/IFN-gamma ratio correlates with clinical response to glatiramer acetate (Copaxone) in multiple sclerosis. Neurology 2005;64(suppl 1):A418. Abstract.