The interplay between the immune and central nervous systems in neuronal injury

ABSTRACT

Once perceived as a region of limited immune activity, the CNS is now known to be an important site of immune interactions. Activated T cells can infiltrate the blood-brain barrier where they accumulate and proliferate in response to antigen restimulation. These leukocytes express proinflammatory cytokines that help in activating microglia and other immune cells. A profound inflammatory response ensues, which can lead to axonal injury and demyelination. In contrast, other T cells can be neuroprotective. CD4+ Th2 cells secrete anti-inflammatory cytokines and can elicit the production of bioactive neurotrophins from CNS glia. In addition, neurons themselves can contribute to immune system regulation by being targets of neurotoxic T cells or by altering T-cell activity, including the generation of regulatory T cells. The interplay between components of the immune system and CNS contributes both to healthy brain function and to the pathogenesis of neurodegenerative diseases such as multiple sclerosis. NEUROLOGY 2010;74 (Suppl 1):S9–S16

GLOSSARY
ARD = autoimmune rheumatic diseases; BBB = blood-brain barrier; BDNF = brain-derived neurotrophic factor; EAE = experimental autoimmune encephalitis; ECM = extracellular matrix; FGF = fibroblast growth factor; HPA = hypothalamic-pituitary-adrenal; IGF = insulin-like growth factor; IL = interleukin; IVIG = IV immunoglobulin; SCI = spinal cord injury; MHC = major histocompatibility complex; MMP = matrix metalloproteinase; M/M = monocytes/macrophages; NT-3 = neurotrophin-3; NO = nitric oxide; OPC = oligodendrocyte precursor cell; PDGF = platelet-derived growth factor; TLR = toll-like receptor; TNF = tumor necrosis factor.

INTRODUCTION
Several features of the CNS had suggested that the cellular environment of the brain is unresponsive to immune activity. These features include the existence of the blood-brain barrier (BBB), low T-cell numbers within the CNS under normal circumstances, graft acceptance, unconventional lymphatics, and reduced major histocompatibility complex (MHC) class II levels.¹ However, much recent research has demonstrated that the CNS is in fact subject to bidirectional immune homeostasis. For instance, it is now known that graft-vs-host disease occurs after brain allografts.² Antigen-carrying dendritic cells emigrating from the brain have been shown to infiltrate peripheral lymph organs, inducing a local immune response and directing antigen-specific T cells back to the brain.³ The addition of interferon (IFN)γ to electrically silent neurons that do not fire spontaneous action potentials has been found to promote the cell surface expression of MHC molecules, which may help in the reactivation of CD8+ and CD4+ T cells within the CNS.⁴ Finally, multiple pathways that facilitate the trafficking of small numbers of unactivated lymphocytes into the CSF or across the blood-CSF-barrier of the normal brain have been identified, as have compensatory mechanisms that ensure BBB integrity.⁵ In short, contrary to conventional thinking, the CNS is a site conducive to immune activity that can, under certain conditions, protect against insults such as cerebral infection or cause inflammatory neurodegenerative diseases, such as multiple sclerosis (MS).

A heightened understanding of the relationship between the immune system and CNS has also spurred interest in the interplay of the neuroendocrine and immune systems. It is now known that the neuroendocrine system can affect the development and function of the immune system, whereas the lat-
ter plays an important regulatory role in the activity of the former. Of greatest relevance here, the hypothalamic-pituitary-adrenal (HPA) axis may play a key role in the pathogenesis of autoimmune rheumatic diseases (ARD) and MS. Interactions among the HPA axis, the hypothalamic-pituitary-gonadal axis, the hypothalamic-pituitary-thyroid axis, the prolactin-gonadal axis, and the immune system are abnormal in patients with ARD, particularly during periods of active disease. Experiments using an animal model of MS, experimental autoimmune encephalitis (EAE), and clinical studies in patients with MS suggest that impaired HPA function may contribute to the onset and severity of the disease. Hyper- and hypoactivity of the HPA axis appear to be associated with both disability and MRI measures of atrophy. Although the precise mechanisms involved in these actions are not well understood, emerging evidence indicates that dysregulation of the HPA axis after physiologic stress, which causes aberrant corticosteroid and immune responses, may influence the etiology, pathophysiology, and course of MS. Thus, the recognition that the CNS is not necessarily an immunoprivileged site has led to a revamping of our understanding of diseases such as MS. This article reviews the interplay between the CNS and the immune system in MS and related conditions. It describes how such interactions can lead to either neuronal injury or repair, partly influenced by the subsets of lymphocytes that are involved. The review continues with an examination of the differential effects of immune cells on those of the CNS, showing several novel types of CNS-immune system engagements, including those after traumatic spinal cord injury (SCI) in mice. The aim is to show that the relationship between the CNS and immune system is far more dynamic and complex, and has far greater clinical implications, than traditional thinking would lead one to predict.

**MULTIPLE ASPECTS OF IMMUNE SYSTEM-CNS INTERACTIONS**

**Neurogenesis.** Neurogenesis in the hippocampus occurs continuously in adulthood and is essential to the performance of cognitive functioning. In normal physiology, potentially autoreactive T cells have been reported to be fundamental to preserving the integrity of the healthy cellular environment of the brain. These leukocytes help maintain homeostasis in the CNS by promoting postinjury neuronal survival and recovery even as, under different conditions, unregulated CNS antigen-reactive T cells have the potential to contribute to the onset of autoimmune diseases such as MS (see later and figure 1). Using immune-deficient mice, Ziv et al. showed that cross-talk between autoreactive T cells in the CNS and local microglia contributes to adult brain plasticity by promoting neurogenesis in the dentate gyrus and subventricular zone. Although hippocampal neurogenesis was severely impaired in T-cell–deficient mice, the replenishment of antigen-recognizing T cells reactive to such peptides as myelin basic protein (MBP) restored the number of adult neurons and spatial learning. Another group found that the T-cell cytokine, IFNγ, facilitated the generation of new neurons in the dentate gyrus of adult mice. These findings demonstrate why immune system dysfunction can have serious effects on brain performance. Furthermore, the age-related loss of certain cognitive functions might partially be a consequence of immune system aging, although a causal connection remains to be established. It also may explain why HIV-AIDS and other conditions stemming from immune compromise are associated with cognitive impairment. In short, the link among the adaptive immune system, lifelong neurogenesis, and cognitive functions such as spatial learning and declarative memory offers a mechanism by which fluctuations in bodily physiology can affect functions associated with the mind.

**Axonal injury.** As noted earlier, autoreactive T cells play an important part in the etiology of MS, a disease characterized by demyelination and axonal loss. Axonal injury occurs early in the course of MS and correlates with the degree of inflammation in the brain. One hypothesis of MS pathogenesis suggests that activated T cells migrate through the BBB where they accumulate and proliferate because of antigen restimulation and release a host of proinflammatory molecules, which, in

![Figure 1](https://example.com/figure1.png)

This figure suggests that several consequences are possible when leukocytes encounter neurons; the latter themselves are influenced through interactions with glia. Treg = T-regulatory cell.
turn, further activate microglia or infiltrated macrophages and B cells. This chain of events produces a marked inflammatory response, which causes axonal injury and demyelination through various antigen-specific and bystander mechanisms.13,14

The means by which T cells induce neuronal toxicity are incompletely understood. Besides injurious proinflammatory molecules, proapoptotic factors produced by T cells, including FasL, granzyme B, soluble TRAIL, and free radicals, are possible mediators of injury.15–18 Giuliani et al.19 observed that both CD4+ and CD8+ T-cell subsets, once activated, are highly neurotoxic. These effects are mediated through a variety of contact-dependent mechanisms involving cell surface molecules such as FasL, LFA-1, and CD40. MHC class I does not appear to be required once T cells have been activated.19 More recently, it has been found that the Th1 and Th17 proinflammatory classes of CD4+ T cells are neurotoxic, whereas the anti-inflammatory Th2 subset is not; indeed, Th2 cells may protect neurons against Th1 toxicity (Giuliani et al., manuscript in preparation). Others have reviewed the data that Th2 cells are beneficial to the CNS.20

Although activated T cells can clearly harm neurons, the converse has also been observed. Flugel et al.21 reported that activated T cells underwent apoptosis that was mediated through neurons through a FasL-dependent mechanism. In another context, neurons may induce encephalitogenic T cells to convert to T-regulatory cells (figure 1) that inhibit encephalitogenic T-cell action and suppress EAE.22 Thus, understanding the conditions by which neurons enhance the death of potentially damaging leukocytes, or alter their characteristics rather than being subjected to their toxicity, is an important step toward improving CNS well-being.

**Protective autoimmunity.** However, not all proinflammatory subsets are neurotoxic. Through a process referred to as “protective autoimmunity,” some T cells offer survival benefits by promoting axonal repair and neuronal recovery.23 In this regard, the injection of myelin-reactive T cells into rodents with SCI24 or optic nerve crush25 ameliorates neuronal and axonal loss that results from the trauma. Using a spinal cord contusion model, Kigerl et al.26 found that locomotor recovery is impaired in mice deficient for toll-like receptor (TLR) 2 and 4 compared with wild-type controls; TLR signaling is vital for initiating innate immune responses such as those provided by the activation of microglia and macrophages in the injured CNS.

**Axonal regeneration.** Neuroinflammation may also promote axonal regeneration. The injection of activated macrophages27 or microglia28 into the lesioned spinal cord enables some degree of axonal regeneration. The capacity of activated macrophages to promote axonal regeneration after optic nerve injury was attributed to a molecule, oncomodulin.29 Others described that stimulation of TLR2 and TLR4 orchestrates the innate immune response to rapidly clear inhibitory myelin debris to allow subsequent nerve regeneration.30

The mechanisms by which antigen-reactive T cells or other leukocyte subsets confer axonal repair or neuronal recovery remain speculative, although leukocytes are known to express bioactive neurotrophins, including brain-derived neurotrophic factor (BDNF) and neurotrophin (NT)-3,31,32 These are important survival and regenerative factors for neural cells during development and in adulthood. Other mechanisms will be discussed in the next section.

**Immune-mediated remyelination.** A recent review33 notes that the repair of CNS damage may require the collaboration of multiple immune system components. Studies in animals have demonstrated that remyelination is impaired after a demyelinating injury in animals deficient in various leukocytes, including T cells,34 macrophages,35 tumor necrosis factor (TNF)α,36 and matrix metalloproteinases (MMPs).37 For instance, transgenic mice lacking both B cells and T cells, which underwent lysolecithin-induced demyelination in the spinal cord showed significantly reduced spontaneous remyelination, compared with controls.34 Similarly, macrophage depletion after lysolecithin-induced demyelination of the spinal cord limited oligodendrocyte remyelination in young female mice.35 Further study showed that remyelination followed a distinctive pattern in which new myelin sheaths and mRNA of the major myelin protein genes began to appear about 10 days after lesion induction and continued to increase until day 21. Mice that were made macrophage deficient only in the late phase of remyelination (i.e., when oligodendrocyte progenitors mature into myelin sheath-forming oligodendrocytes) showed a similar pattern to control animals, whereas those that were depleted of macrophages during the early phase (i.e., when oligodendrocyte progenitors are recruited) differed significantly from controls. These results indicate that macrophages are required for remyelination during the recruitment period of oligodendrocyte lineage cells, whereas their presence during the maturation phase provides no advantage.35

In corroboration of these depletion studies, the promotion of local neuroinflammation can lead to more efficient repair. A mechanical lesion injury38 to the spinal cord or the local application of zymosan (a TLR2 agonist)39 both of which increased the extent
of inflammatory responses within the spinal cord, facilitates remyelination in animals.

There are multiple ways by which immune cells may promote CNS recovery. These include a) clearance of debris such as myelin fragments or toxic substances such as β-amyloid through phagocytosis; b) promotion of a beneficial extracellular matrix (ECM) environment; c) production of proteases (e.g., MMP-2 and MMP-9) that regulate axon morphology and growth and help remove proteoglycans that inhibit remyelination; and d) secretion of assorted neurotrophic factors.37-40-43 Bone marrow-derived microglial cells in adult mice phagocytosed amyloid deposits in the brain, thereby preventing the formation of amyloid plaques, the signal feature of AD.41 These newly recruited microglia had a stronger chemotaxis to the protein than resident cells and thus appeared to be more efficient immunomodulators of disease progression.41 In a similar fashion, macrophages are known to be responsible for debris clearance after demyelination, a process essential to oligodendrocyte precursor cell (OPC) differentiation and myelin repair.45 Macrophage-depleted mice showed an impaired response to inflammation and a delayed removal of myelin debris. In addition, disruption of normal macrophage activity altered the lesion-signaling environment, downregulating the expression of growth factors important for remyelination, including insulin-like growth factor (IGF)-1, transforming growth factor-β1, and trophic factors necessary for the survival of OPCs and differentiated oligodendrocytes.46 Finally, mixed splenocytes and T and B lymphocytes obtained from mice expressed mRNA for a wide range of neurotrophins, including nerve growth factor (NGF), BDNF, and neurotrophin (NT)-3.43 Moreover, B and T cells themselves were capable of producing substantial levels of NGF and NT-3.

T-cell interactions with microglia. Glial cells have been implicated in the pathogenesis of many neurodegenerative diseases, including MS. Activated microglia and astroglia can express a number of neurotoxic molecules, including TNFα, nitric oxide (NO), and various interleukins (IL). Dasgupta et al.44 showed that neuroantigen-primed T cells, in particular those recognizing MBP, promote glial cell expression of proinflammatory enzymes such as inducible NO synthase and cytokines (IL-1β, IL-1α, IL-6, and TNFα) by cell-to-cell contact. However, activated glial cells also have the potential to release neurotoxins, although the molecular pathways that shift the direction of glial activity from a proinflammatory to an anti-inflammatory course have not been well defined. Recent experiments by Roy et al.45 suggest that cell-cell contact between MBP-primed Th2 cells and microglia or astroglia promotes the expression of BDNF and NT-3 without inducing release of the aforementioned proinflammatory cytokines. Intercell contact between integrins on the T-cell surface and platelet-derived growth factor (PDGF)-β on the glial cells help mediate neurotrophin expression. Moreover, these MBP-primed Th2 cells migrate into the CNS where they upregulate neurotrophin release throughout the brain environment. In contrast to the Th2 cells, the group found that Th1 cells engaged glia to produce proinflammatory cytokines rather than neurotrophins.45

Thus, Th1 and Th2 cells produce differential effects on glial cell activity and gene expression. MS lesion development may be contingent on the balance among Th1 cells, monocytes/macrophages (M/M), and Th2 cells in the CNS. Lisak et al.46-48 undertook a series of experiments to try to determine how different mixtures of cytokines affect glial cell gene expression. In the first experiment, rat glial cells were incubated with mixtures of Th1, M/M, and Th2 cells for 6 hours and were then examined using microarray gene chip technology for changes in early gene expression.46 The various immune cell mixtures generated a large number of alterations in the mixed glial cell cultures.46 Compared with controls, >800 genes showed significant differences in response to at least 1 of the mixtures (p < 0.05), with more genes being downregulated than upregulated. Th1 cytokines had a marked upregulatory effect on gene expression of MHC class I and II molecules, whereas Th2 cytokines downregulated some of these molecules. Th1 cytokines promoted cell adhesion and increased expression of ECM molecules, whereas Th2 cytokines had limited effects. The different cell mixtures also generated a complex and differential pattern of gene regulation of MMPs, cytokines, other surface proteins, and complement pathway components.

Subsequent studies demonstrated that the products produced from the unique cell mixtures induced unique alterations in genes affecting neuroprotection and axon/glial relationships, as well as CNS glia metabolism, signaling, and regulation.47,48 More specifically, Th1 cytokines regulated genes for IGF-1 and insulin-like growth factor receptor-5, M/M cytokines regulated those fibroblast growth factor (FGF)-2 and FGF-5, and only Th2 regulated FGF-14, neuregulin 1, IGF-2, and PDGF receptor α.47 Th2-derived molecules also uniquely controlled expression of a large number of genes for adhesion/ECM molecules, whereas Th1 and M/M cytokines regulated more neuronal-related peptides than did Th2 cytokines. Furthermore, even though most genes for neurotrophins and their receptors were downregulated by the several mixtures, Th2 cyto-
kines were found to induce a 2-fold greater expression of the genes that regulated the expression of BDNF. Finally, all 3 cytokine mixtures downregulated the dopamine D3 receptor, whereas Th1 and Th2 inhibited expression of neuropeptide Y receptor-5. Most surprising were the large number of changes related to lipid metabolism. This body of research, which demonstrated that glial gene transcription is altered profoundly within 6 hours of cytokine exposure, identified microglia and astroglia as primary actors in the amplification or suppression of toxic activity in the brain. Further research will help clarify which glial type is responding and whether the aforementioned changes lead to long-lasting alterations in gene expression and function and in immune system interactions.

Our group has closely investigated the scientific and clinical implications of the T cell-microglial relationship. Initial experiments demonstrated that activated human T leukocytes induce the microglial production of the proinflammatory cytokine TNFα, a key regulator of immunologic activity in the CNS. In culture, adult human microglial cells assume different morphologies, although the majority is elongated in shape. However, when cocultured with activated T cells, microglia became amoeboid in appearance, indicating a state of activation. After 24 hours, this T-cell–activated medium contained significantly greater amounts of TNFα, compared with isolated cultures of either microglia or T cells. The mechanism for this cytokine expression was subsequently found to involve the integrin very late antigen-4 on T cells and its ligand vascular cell adhesion molecule-1 on microglia (figure 2). The presence of TNFα in the CNS of MS may be contributed significantly by T-cell infiltration into the CNS where these lymphocytes come into close proximity with microglia, leading to TNFα expression and oligodendrocyte injury.

A similar pattern was established for IL-10, a cytokine with anti-inflammatory properties. Although neither microglia nor T cells alone release substantial amounts of IL-10, coculturing the 2 induced significantly higher levels of the cytokine, when compared with culturing microglia alone. Both cells were active in IL-10 expression, which was dependent on a cell-to-cell contact mechanism involving the CD40, B7, and CD23 pathways (figure 2). We also found that CD4+ and CD8+ T cells are responsible for the production of both IL-10 and TNFα in microglia-T-cell cocultures. Whether the 2 cytokines, one of which is predominantly proinflammatory and the other predominantly anti-inflammatory, can be regulated selectively is an important question that emerged from the research. The answer may have important implications for treatment (figure 3).

Indeed, we have noted that one of the hallmarks of contemporary immunomodulatory therapies for MS is attenuation of the microglia-T-cell dynamic. We have established that glatiramer acetate reduces levels of IL-10 and TNFα in adult human T cell-microglia cocultures. This effect is not a consequence of a change in T-cell proliferation or survival nor is it due to altered T-cell adherence with microglia. Although the precise mechanism by which glatiramer acetates prevent “modulated” T cells from engaging with microglia remains to be established, the effect is clear: a reduction in the level of both Th1 and Th2 cytokines in the brain environment. Whether the mechanism is related to glatiramer acetate’s ability to generate neuroprotective Th2-reactive cell lines (which cause bystander suppression of cytotoxic Th1 cells) awaits the result of future research.

Figure 2

Cell-to-cell contact between activated T cells and microglia leads to the expression of TNFα (through the binding of VLA-4 and VCAM-1) or IL-10 (through B7, CD40, and CD23 pathways)

Figure 3

Affecting T cell-microglia interaction is a feature of many treatments for MS

GA = glatiramer acetate; IFNβ = interferonβ; HQ = hydroxychloroquine; IVIG = IV immunoglobulin.
The ability of T cells to bind with microglia is also altered by IFNβ, although the pattern differs from that of glatiramer acetate. Although glatiramer acetate downregulates cytokine expression, IFNβ-treated T cell-microglia cocultures increase levels of IL-10 and decrease levels of IL-1β, IL-4, IL-12, IL-13, and TNFα. Levels of IL-6 remain unchanged.

Experiments examining the effects of several other investigational drugs on the coculture of T cell-microglia are briefly worth noting. Minocycline, a tetracycline with anti-inflammatory properties, also reduces expression of TNFα in this medium through the direct action of the drug on the activated leukocytes. In so doing, minocycline reduces the potential for cell-cell contact and the subsequent release of proinflammatory cytokines. Similarly, therapeutic doses (1–5 mg/mL) of the immunomodulator IV immunoglobulin (IVIG), a drug currently used for treatment of autoimmune diseases, alter T-cell reactivity, thereby decreasing the ability of T cells and microglia to interact. Reduced expression of TNFα and IL-10 were observed. Similar results have been observed with hydroxychloroquine (unpublished data).

**Microglia/macrophage activity.** Significant activation of microglia/macrophage is evident in MS, particularly in cases of secondary-progressive MS. Because microglial activation is known to promote an inflammatory response leading to myelin degradation and axonal damage, the study provides further evidence of the large role played by microglia in the pathophysiology of MS.

More specifically, microglia and macrophages have been shown to promote disease activity by regulating both the invasion of autoreactive T cells and the recruitment of secondary glial cells, inhibiting the development and maintenance of inflammatory CNS lesions, upregulating cytokine expression, and timing the induction of the immune response. Inhibition of these activities in EAE suppresses manifestations of disease symptoms. For instance, depleting peripheral macrophages in SJL/J mice by IV injections of mannosylated liposomes containing dichloromethylene diphosphonate prevented the induction of EAE by limiting leukocyte migration and accumulation across the BBB and into the CNS parenchyma. Although macrophage depletion did not alter expression of Th1 cytokines, cytokine secretion by glial cells was prevented, reducing pathology and symptoms. In a similar manner, administration of ganciclovir to CD11b-thymidine kinase transgenic mice produced paralysis of microglia in vivo. This paralysis reduced the levels of microglial-derived proinflammatory cytokines and chemokines and ameliorated the clinical signs of EAE. Overall, persistent CNS microglia/macrophage activity is a likely key contributor to the development of local inflammatory injury, and the timing and intensity of their activation are crucial to the development and progression of autoimmune diseases such as EAE.

**SCI and the early inflammatory response.** Secondary degeneration after SCI involves a complex cascade of events that leads to further tissue damage beyond the initial traumatic event. This cascade includes vascular damage, ischemia/hypoxia, release of free radicals, excitotoxicity, ionic dysregulation, and inflammation, all of which promote neuronal and glial apoptosis, demyelination, and axonal loss. The initial inflammatory response to SCI activates microglia and astrocytes, which release proinflammatory cytokines, chemokines, and lipid mediators that recruit immune cells to the site of injured tissue. During the first hours after injury, SCI also mobilizes circulating neutrophils. These cells migrate to the spinal cord in large numbers where they release a host of neurotoxic substances. As a consequence, efforts have been focused on neutrophil trafficking to modulate secondary degeneration post-SCI. A study conducted by our group, however, produced surprising results: rather than reducing neuronal and axonal injury, neutrophil depletion actually worsened short-term behavioral and histologic outcomes.

Mice exposed to an antibody selected to reduce neutrophil counts (anti-Ly6G/Gr-1) had circulating neutrophil levels reduced by >90%, compared with controls, along with a parallel reduction in neutrophil numbers at the site of cord injury. However, astrocytic reactivity was also reduced relative to controls, with treated mice having an altered growth factor milieu, increased lesion area, less preserved white matter, and diminished wound healing. Behavioral outcomes worsened as well. Thus, neutrophil trafficking may contribute to both neurotoxic and neuroprotective responses to CNS injury or trauma. The probable mechanism for neutrophils’ beneficial effects after SCI involves the promotion of astrocyte reactivity and changes in the growth factor environment, although the precise nature of the interaction remains to be determined.

**CONCLUSION** Recent research into the interplay between the immune system and CNS has yielded a substantial body of evidence demonstrating that the relationship is far more dynamic and complex than previously thought. Heretofore perceived as a site of immunoprivilege, the CNS is, in fact, a region of immune homeostasis. Some activated leukocytes can be highly neurotoxic, whereas others promote neuronal health and recovery. In contrast, brain cells contribute to immune system regulation by potentiating activated T-cell
proliferation and action or they may dampen immune reactivity. In sum, interactions between the immune system and the CNS are essential to the functioning of the healthy brain and one of the keys to understanding the etiology, pathophysiology, and natural history of neurodegenerative diseases such as MS.

DISCLOSURE

Dr. Yong served on scientific advisory boards for Teva Neuroscience and Bayer; received honoraria for consultations from Teva Neuroscience, Novartis, and Merck Serono; accepted honoraria for speaking engagements from Teva Neuroscience, Novartis, Biogen Idec, and Merck Serono; had an operating grant for research from Teva Pharmaceuticals, Israel; and received stock options from Osprey Pharmaceuticals and Stem Cell Therapeutics. Mr. Marks reports no disclosures.

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