ABSTRACT

The infiltration of peripheral blood T-lymphocytes into the central nervous system (CNS) is observed to some degree in most patients with active multiple sclerosis (MS) lesions. Several lines of evidence support a role of T-cells in the development of MS lesions. T-lymphocytes produce several soluble mediators that contribute directly or indirectly to neuronal degeneration. However, many mediators released by T-lymphocytes or other immune cells (e.g., macrophages) have both beneficial and harmful effects in the CNS. Several in vitro methods have been developed to quantify the cellular and molecular mechanisms underlying human neuronal injury. Measures of neuronal death, neurite retraction, oxidative stress, or mitochondrial membrane potential may provide new tools to evaluate potential neuroprotective therapies for MS and other CNS disorders.

Although the clinical and pathologic manifestations of multiple sclerosis (MS) vary considerably from patient to patient, the infiltration of T-lymphocytes into the central nervous system (CNS) occurs to some degree in most patients with active MS lesions. T-cells migrate into the CNS and initiate and amplify cellular signaling cascades that activate cell death pathways. Considerable recent research using in vitro and in vivo experimental models has characterized several soluble mediators produced by T-lymphocytes and other immune cells that contribute to neuronal pathology. These studies have described a series of complex interactions between inflammatory mediators and their cellular targets, with many of these substances producing both beneficial and harmful effects. These complex interactions may make it difficult to significantly improve the course of MS by therapeutic strategies that target only a single mediator or intracellular signaling pathway.

Several lines of evidence suggest that T-cells are important in the pathogenesis of MS. As described by Dr. Raine, T-cells are present at the margins of active MS lesions. The risk of developing MS has been associated with specific class II major histocompatibility complex (MHC) molecules suggesting an important role for CD4+ T-cells. Myelin basic protein (MBP)-specific T-cells in peripheral blood display increased rates of somatic mutation and memory. Clinical trials examining the modification of immune activity have also provided circumstantial evidence regarding the importance of T-lymphocytes in MS. In the Altered Peptide Ligand (APL) trial, patients with MS were treated with a peptide designed to partially mimic the encephalitis-producing epitope of MBP with the goal of blocking T-cell responses to MBP or shifting T-cell activity to a regulatory profile. However, the status of several patients who received the peptide worsened dramatically, with increased numbers of contrast-enhancing lesions and clinical exacerbations. Cross-reactivity was demonstrated between the APL and MBP, as well as the expansion of MBP-reactive T-cells. Instead of APL acting as a weak agonist and driving a TH2 response as intended, the APL signaled T-cell receptors and activated TH1 myelin-reactive cells, which led to a marked increase in the number of active enhancing lesions in some patients.

These findings suggest that T-lymphocytes are intimately linked to the initial inflammatory demyelination in MS. Recent evidence suggests that T-cells might also mediate direct damage to neurons and axons. It is widely believed that therapies targeting the

CYTOPATHES AND OTHER INFLAMMATORY MEDIATORS OF AXONAL INJURY*

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harmful actions of T-lymphocytes could reduce the severity or rate of progression of MS.

**Chemical Mediators of T-Cell Activity**

T-cells may play an early role in the pathologic processes in MS through cross-reactivity between microbial antigens, such as Epstein-Barr virus and encephalitogenic myelin epitopes. This process probably begins at such a low level that early pathologic changes are undetectable by conventional magnetic resonance imaging. Recent studies have revealed spectroscopic and magnetization transfer ratio abnormalities in normal-appearing white matter months before inflammatory lesions (see article by Dr Frank). It remains possible that an intrinsic lesion in the CNS white matter (infection or myelin biochemical abnormality) precedes any inflammatory process.

Activated T-cells traffic continually through the subarachnoid perivascular space. Whether these T-cells migrate into the CNS parenchyma or recirculate back into cervical lymphatics may be regulated by chemical mediators known as chemokines and whether that T-cell's cognate antigen is present. Activated T-cells express specific chemokine receptors that are sensitive to gradients of chemokine concentration and guide the migration of the cells into the CNS. Chemokines are released by both astrocytes and activated endothelial cells. Targeting effector T-cells may be an effective strategy to reduce neurologic injury in MS. One approach to targeting these cells is by using monoclonal antibodies that target the alpha-4 chain of the very late antigen-4 dimer, getting these cells is by using monoclonal antibodies that reduce neurologic injury in MS. One approach to targeting effector T-cells may be an effective strategy to reduce neurologic injury in MS. One approach to targeting these cells is by using monoclonal antibodies that target the alpha-4 chain of the very late antigen-4 dimer.

**CHEMICAL MEDIATORS OF T-CELL ACTIVITY**

Chemokines are secreted by macrophages, including IL-1-beta, IL-18, IL-12, and IL-23. Some cytokines, such as IL-10 and transforming growth factor-beta, can be secreted in significant quantities by either dedicated regulatory T-cells or macrophages.

Although cytokines are often described as either generally beneficial or harmful in a particular illness, it is important to note that many cytokines have dual roles. TNF-alpha has a clear role in the induction of inflammatory cytokines such as IL-1-beta and IL-6, and induction of adhesion molecules on the vascular endothelium by which T-cells migrate into the parenchyma. TNF-alpha also increases the release of reactive oxygen species, suppresses glutamate reuptake by astrocytes, and contributes to the death of oligodendrocytes. Moreover recently, however, TNF-alpha has been implicated as a neuroprotective agent that is important in neuron survival and differentiation. Antagonism of TNF-alpha was shown to worsen or mediate demyelinating disease as was seen in a trial of a soluble TNF receptor antagonist in MS. There are also several reports of CNS demyelination in patients taking TNF-alpha antagonists to treat rheumatoid arthritis. IL-6 also appears to have a significant dual role in CNS injury. Some evidence suggests that IL-6 possesses neuroprotective effects. It also inhibits the expression of cellular adhesion molecules and promotes the expression of nerve growth factors. IL-6 has been shown to reduce neurologic injury in experimental allergic encephalomyelitis (EAE), an animal model of immune-mediated demyelination that is thought to be a model of MS. It is also a potent proinflammatory agent that is produced in large quantities by astrocytes and is found in the cerebrospinal fluid following inflammation. IL-6-deficient mice are resistant to the myelin oligodendrocyte glycoprotein-induced EAE, but the addition of IL-6 in the preclinical phase allows for disease to develop. In patients with transverse myelitis, the concentration of IL-6 in cerebrospinal fluid has been shown to correlate with patient scores on the Expanded Disability Status Scale (D. Kerr, oral communication).

Although the immune response in MS is usually considered an adaptive response with secondary activation of T-lymphocytes, innate immunity may also be significant in the pathogenesis of MS. Neuronal death may cause the release of substances (eg, heat-shock proteins) that stimulate the activation of microglia. This interaction takes place through a specific microglia receptor, known as the toll-like receptor (TLR). Activation of the TLR may also occur in other neurodegenerative disorders (eg, Alzheimer's disease, Parkinson's disease), although the microglia activation is probably...
not as extensive. Nonspecific infections may also activate microglia or macrophages through the TLR. Infections may also produce other activating effects that are important in the pathogenesis of MS. Some infectious agents (eg, Epstein-Barr virus) cause transactivation of human-associated retrovirus, which stimulates the activation of T-cells secondarily through an effect on superantigens. Infection may also cause direct toxic effects on neurons, although the evidence for this is more limited.

**Pathways of T-cell-Mediated Cell Death**

Many cellular signaling pathways can produce cell death. Two pathways that may be particularly important in MS are T-cell-mediated cell killing via members of the TNF receptor family and the granule-mediated cell death pathway.

The TNF receptor family includes the Fas receptor on the T-cell and the Fas ligand on the antigen-presenting cell. Activation of the Fas receptor by the Fas ligand causes the activation of a cascade of intracellular caspasess (substances that cleave aspartic acid), which in turn trigger apoptotic cell death pathways (Figure 1). This system is regulated by a Fas ligand inhibitory protein (FLIP). The Fas receptor is found in large numbers on some antigen-presenting cells, and the FLIP prevents these cells from mutually triggering cell death pathways in each other.

T-cell release of cytotoxic proteases (granule release) is a second important pathway of T-cell-mediated death. The principal molecules in this process are perforin and granzyme (Figure 2). Perforin is transported to the surface of the T-cell where it acts at the interface between the T-cell and its target. This enzyme perforates the membrane of the target cell, which allows the entry of granzyme into the cell. Recent data suggest perforin may also act intracytoplasmically. Granzyme is initially inactive and is converted to an active form by cathepsin G to a proteolytic enzyme. Granzymes were long thought to act by a caspase-dependent pathway; more recently, however, it has become clear that there are also caspase-independent pathways by which granzymes damage mitochondrial membranes.

Another group of proteases with an important role in axonal injury is the matrix metalloproteases (MMPs). MMPs target extracellular matrix proteins, which are important in cell migration, metastasis,
wound healing, and angiogenesis. MMPs have some beneficial effects on CNS function (eg, they are important in establishing an environment that is conducive to cell growth). Some MMPs are also important in inflammation, especially MMP-2 and MMP-9, which are secreted by leukocytes in order to facilitate proteolysis of membrane proteins during migration. MMP-1, which is produced by activated astrocytes, increases the expression of MMP-9, which in turn can cleave MBP in addition to mediating degradation of the blood-brain barrier. MMP-1 has been shown to be directly toxic to cultured neurons (Figure 3). Microinjection of other activated MMPs (MMP-2, MMP-7, or MMP-9) into the cortical white matter of rats caused significant neuronal degeneration, with the most extensive injury following microinjection of MMP-9.

Several other proteases may also be important in cell death pathways. Cathepsin E and S are endosomal/lysosomal proteases that are important in MHC-II mediated antigen presentation of microglia. Tissue-type plasminogen activator is a serine protease that participates in neuronal death and the activation of microglia. Calpain is a calcium-dependent cysteine protease that participates in the extracellular degradation of myelin.

**Modeling Neurotoxicity**

Several in vitro and in vivo model systems have been developed to examine the cellular and molecular processes of neuron death. Human fetal neurons can be grown using cell culture techniques, and neuronal pathology or cell death can be evaluated by staining cells for specific markers. Microtubule-associated protein-2 staining and computer-assisted measurement allow quantification of neurite growth or retraction; trypan blue or annexin V provide markers of neuronal death; and dihydroxyrhamdamine is a marker of oxidative stress. Cytochrome c release has been used as a marker of mitochondrial injury following exposure of cultured fetal neurons to cerebrospinal fluid from HIV-infected patients with dementia. These methods have been used to study neuronal injury as a consequence of HIV infection, and similar methods are being applied to the study of T-cell-mediated neuronal injury that may occur in MS. Giuliani and colleagues reported that activated T-cells are directly toxic to neurons but not to other CNS cell types (eg, oligodendrocytes, astrocytes). These observations have been extended by studies showing that exposure of cultured neurons to both activated CD4+ and CD8+ T-lymphocytes reproducibly causes mitochondrial membrane potential changes, neurite retraction, and eventually neuronal death (P. Calabresi et al, unpublished observations). This T-cell-neuronal coculture system may allow further characterization of T-cell-secreted neurotoxins, a better understanding of the molecular pathways involved in human neuronal death, and a way of screening for potential neuroprotective compounds.

**Summary and Conclusions**

T-lymphocytes and other immune cells infiltrate into the CNS and contribute to the inflammatory processes that underlie active MS lesions. These cells produce several inflammatory and regulatory mediators that mediate neuronal dysfunction and death. Many of these inflammatory proteins have dual roles and may act as either neuroprotective or neurotoxic agents in different settings. Several experimental systems have been developed to further characterize the role of T-lymphocytes and other inflammatory cells in the pathogenesis of MS and to evaluate new potential therapeutic strategies for MS and other disorders of the CNS.

**Figure 3. LDH Release as Index of Neuronal Damage by MMP**

LDH release was used as an index of neuronal damage in cultured neurons treated with MMP-containing buffers. Exposure of the cells to MMP-1 markedly increased LDH activity. Mean ± SEM of 3 determinations for each MMP. LDH = lactic acid dehydrogenase; MMP = matrix metalloproteinase. Adapted with permission from Vos et al. Cytotoxicity by matrix metalloproteinase-1 in organotypic spinal cord and dissociated neuronal cultures Exp Neurol. 2000;163:324-330. Copyright © 2000, with permission from Elsevier.
REFERENCES


