Cytokines in the brain during viral infection: clues to HIV-associated dementia.

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Perspective

Perspectives Series: Cytokines and the Brain

Cytokines in the Brain during Viral Infection: Clues to HIV-associated Dementia

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Immune responses induced by virus infections of the central nervous system

Control of virus infection of the central nervous system (CNS)[1] is a complicated task for the host because of the potentially damaging consequences of immune responses in the brain and spinal cord. Neurological damage can be associated with cellular cytotoxicity, altered vascular permeability, and the influx of inflammatory cells into an enclosed space containing nonrenewable cells of vital importance to the host. Not surprisingly, therefore, the CNS has a number of mechanisms for controlling and regulating the development of local inflammatory processes. These include the blood-brain barrier, limited capability for antigen presentation, and functional modulation of immune reactions by gangliosides and astrocytes.

The blood-brain barrier is composed of nonreactive endothelial cells linked by tight junctions, a basement membrane, and apposing astrocytic foot processes (see Licinio perspective, Fig. 1, in this issue of *The Journal*). This barrier effectively excludes most circulating soluble factors and circulating leukocytes from the parenchyma of the brain and spinal cord. In addition, cells within the CNS do not normally express class I or class II antigens of the MHC, which are necessary for recognition of antigens by T cells. However, T cells activated in the periphery do cross the blood-brain barrier and enter the CNS in an antigen-nonspecific manner as a part of routine immunologic surveillance. These activated T cells express the adhesion molecules and matrix metalloproteases necessary to traverse resting cerebral capillary endothelial cells and the basement membrane. Only T cells specific for an antigen present in the brain or spinal cord are retained within the CNS. Cells without such specificity leave within a few hours (1). Antigen-presenting cells which promote retention of specific lymphocytes within the CNS are most likely to be perivascular macrophages or microglial cells, both of which can quickly upregulate MHC antigen expression in response to stimulation.

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1. *Abbreviations used in this paper:* CNS, central nervous system; CSF, cerebrospinal fluid; LCMV, lymphocytic choriomeningitis virus.

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After activated T cells enter the CNS and interact with local antigen-presenting cells, production of cytokines will amplify the inflammatory response. However, the activity of these antigen-specific T cells is strongly regulated by the high concentrations of gangliosides present in the CNS. Gangliosides inhibit T cell proliferation and production of IL-2 after entry into the CNS without decreasing production of the type 2 cytokines IL-4 or IL-10 (2). In addition, astrocytes inhibit production of IL-12, necessary for induction of a type 1 cytokine response (3). These and other data suggest that the CNS, more than other organs, favors the development of local immune responses characterized by the production of type 2 cytokines by T cells (4). These cytokines favor the differentiation of B cells for local antibody production and downregulate macrophage activation by IFN-γ. Failure of immune regulation within the CNS due to the genetic background of the host or the nature of the stimulus would favor the development of autoimmune neurologic disease which is generally associated with production of type 1 cytokines or a response to infection that is detrimental to the host.

The potential sources of cytokines in the brain are the intrinsic cells of the CNS (neurons, astrocytes, oligodendrocytes, and microglia) and infiltrating inflammatory cells from the periphery ($CD4^+$ and $CD8^+$ T lymphocytes, B lymphocytes, monocyte/macrophages, and natural killer cells). Several studies suggest that intrinsic cells of the CNS can produce IL-1β, IL-6, IL-10, and TNF-α. These cytokines are all produced by cells of macrophage lineage and are likely to be the products of activated microglial cells and perivascular macrophages. Although neurons, oligodendrocytes, and astrocytes can be induced to produce a variety of cytokines in vitro, their contributions to the in vivo response have been less clear. Overproduction of TNF-α or IL-6 in the CNS of transgenic mice leads to progressive neurodegenerative disease, suggesting that regulation of synthesis of these and likely other proinflammatory cytokines is important for preventing immune-mediated CNS damage (5, 6).

Cytokine responses in acute virus infections of the CNS with fatal and nonfatal outcomes

Despite the checks on detrimental inflammatory responses and cytokine production in the CNS, there are a number of examples of immunologically mediated neurologic diseases associated with type 1 CD4 T cells (e.g., experimental autoimmune encephalomyelitis) and with CD8 cytotoxic T cell responses to virus-infected neural cells. The best characterized virus disease in this category is fatal choriomeningitis of mice induced by infection with lymphocytic choriomeningitis virus (LCMV). LCMV is noncytopathic for the cells it infects, so tolerant animals become infected, but do not develop neurologic disease. However, in immunocompetent adult mice virus-specific cyto-

Table I. Comparison of the Immune Response during Fatal (e.g., LCMV) and Nonfatal (e.g., Alphavirus) Acute Viral Infections of the CNS in BALB/c Mice

	Fatal CNS infection	Nonfatal CNS infection
Inflammatory cells	$CD8^+ > CD4^+$ T cells, macrophages	$CD4^+ > CD8^+$ T cells, macrophages, B cells
Cytokines	IFN-γ > IL-4	IL-4 > IFN-γ

toxic $CD8^+$ T cells and macrophages infiltrate the choroid plexus and meninges a few days after infection. The chemokine IP-10 and IFN-α/β are produced by resident CNS cells early after infection and the infiltrating CD8 T cells produce IFN-γ (7). These $CD8^+$ cytotoxic T lymphocytes simultaneously clear virus and cause fatal disease within a week of infection by a perforin-dependent mechanism. Soluble factors undoubtedly play a role in induction of this inflammatory disease, but fatal disease appears to be mediated by direct killing of LCMV-infected cells by $CD8^+$ cytotoxic T cells (8).

In humans, tropical spastic paraparesis caused by persistent infection with human T lymphotropic virus type-1 (HTLV-1) is also strongly associated with infiltration of the spinal cord with activated HTLV-1–specific $CD8^+$ T lymphocytes. These T cells produce IFN-γ, TNF-α, chemokines, and matrix metalloprotease when studied in vitro (9). The data suggest that immune responses in the human CNS can also mediate disease.

In nonfatal viral encephalitis in mice, a different type of inflammatory response and cytokine environment is present in the CNS (Table I). Alphavirus infection of neurons induces early local expression of IL-1β, IL-4, IL-10, and TNF-α and infiltration of $CD4^+$ T cells followed by macrophages, B cells, and $CD8^+$ T cells (10, 11). The influx of inflammatory cells further augments the levels of these cytokines and adds T cell cytokines such as IFN-γ to the local reaction. In alphavirus infection, virus replication is completely controlled, but virus-infected cells are not eliminated, by virus-specific antibody (12). This noncytotoxic mechanism of control allows for complete recovery of the host and there is no evidence of long-term cytokine-mediated CNS damage.

Immunologic insights into HIV-associated dementia

It is generally agreed that neuronal dysfunction in HIV-associated dementia occurs by an indirect mechanism. HIV-infected cells in the CNS are almost exclusively of macrophage lineage: microglia, resident and infiltrating macrophages. Astrocytes and endothelial cells may occasionally be infected, but appear to produce little virus and to be a very small percentage of all infected cells. Essentially all patients with HIV infection have CNS infection, but only ~ 30% ever develop dementia, and the amount of virus in the CNS is not a good predictor of this complication. The best pathologic correlate for HIV-associated dementia is an increase in the number of activated macrophages in the white matter (13). By immunohistochemical analysis, these cells show increased expression of MHC antigens, TNF-α, TGF-β, IL-1β, IFN-α, nitric oxide synthase, and TNF receptors (14, 15). Two approaches have been used to identify the differences in the CNS between HIV-infected patients with and without dementia: measurement of mRNA and protein expression in brain homogenates at autopsy and measurement of soluble factors in cerebrospinal fluid (CSF).

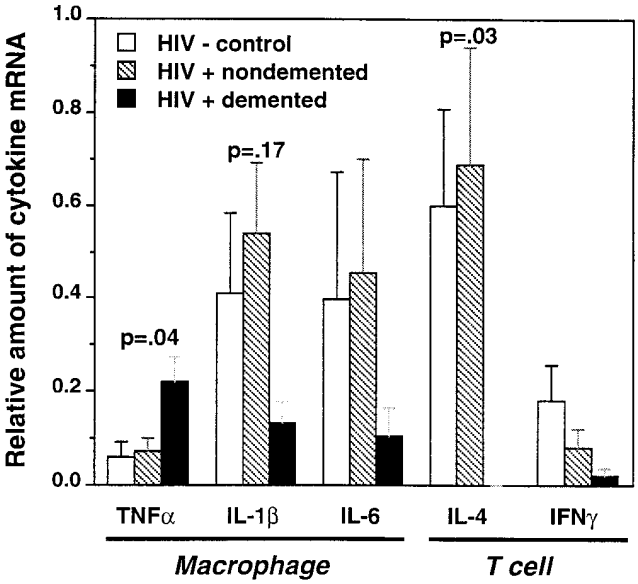

Figure 1. Levels of cytokine mRNAs in the deep white matter of HIV-infected individuals with and without dementia and controls. Levels of TNF-α mRNA are increased while other macrophage-produced cytokines are not. IL-4 mRNA is absent from the CNS of individuals with HIV-associated dementia. Data from reference 16.

Levels of cytokine mRNAs in autopsy-collected brain tissue of individuals with premortem neurologic evaluation have been assessed by semiquantitative RT-PCR for cytokines produced primarily by macrophages. TNF-α and inducible nitric oxide synthase mRNAs are significantly elevated while IL-1β is decreased in demented compared with nondemented HIV-infected individuals (Fig. 1) (16). Furthermore, the levels of TNF-α mRNA increase with increasing severity of dementia. No differences in levels of IL-6, leukemia inhibitory factor, TGF-β, or monokine induced by gamma interferon-2 mRNAs were detected between demented and nondemented patients. TNF-α is found in brain homogenates from HIV-infected individuals (17) and combined in situ RT-PCR and immunocytochemistry has confirmed that macrophages are the source of this cytokine (18).

These studies demonstrated that elevated TNF-α mRNA is an important indicator of dementia, but the direct role of TNF-α in inducing neuronal dysfunction is less clear. The lack of an increase in other macrophage products suggests a relatively specific upregulation of synthesis of this cytokine. TNF-α enhances HIV replication by activating NFκB, an important transactivator of HIV transcription. Oligodendrocytes appear to be susceptible to TNF-induced programmed cell death, but astrocytes are induced to proliferate (19, 20). TNF-α has little effect on neurons in culture, but in vivo TNF induces neuronal injury possibly by inhibiting glutamate uptake by astrocytes (5, 21–23).

When T cell cytokine mRNAs were assessed, the most dramatic difference was in levels of IL-4. IL-4 mRNA was not detected in the brain of any patient with dementia while most nondemented HIV-positive and HIV-negative patients have IL-4 mRNA easily detectable (Fig. 1). IFN-γ mRNA was present at similar levels in demented and nondemented indi-

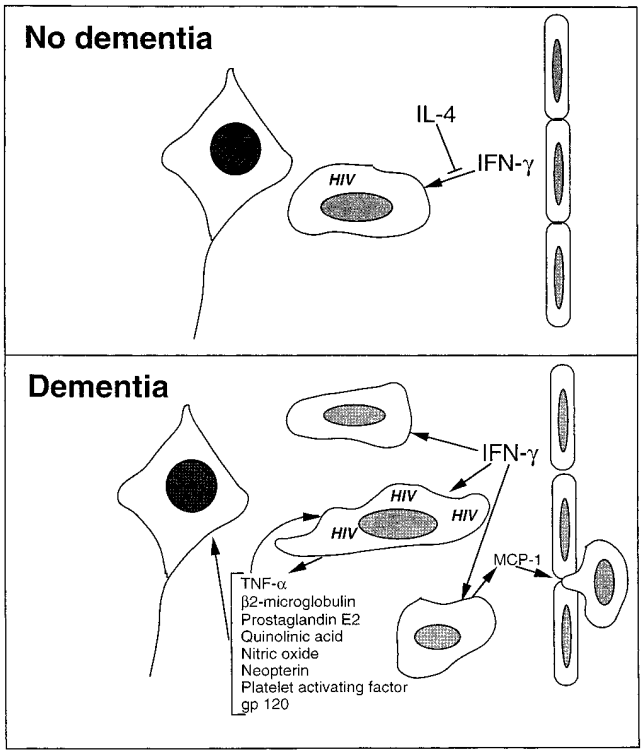

Figure 2. Hypothesized determinants of uncontrolled macrophage activation and dementia during HIV infection. Individuals who develop dementia have no local production of the downregulating cytokine IL-4 leading to uncontrolled activation of macrophages, blood-brain barrier dysfunction, accumulation of macrophages in the CNS, and production of potentially toxic macrophage products.

viduals while IL-2 mRNA was rarely detected (16). IL-4 suppresses macrophage activation by IFN-γ, increases synthesis of nerve growth factor by astrocytes and protects neurons from the cytotoxic effects of activated microglia (24–26). Therefore, the loss of IL-4, and perhaps other macrophage downregulatory, and potentially protective, T cell factors in demented patients may contribute to continued macrophage activation and neurotoxicity. These data suggest that an important determinant of susceptibility to HIV dementia is the inability to produce the downregulatory Th2 cytokine IL-4 (Fig. 2).

Studies of CSF have the decided advantage over examination of nervous system tissue at autopsy in that they can be performed sequentially while the patient is still alive. Changes in CSF can then be associated with the progressive development of dementia and severity of neurologic dysfunction at several points in time. Several soluble factors, such as IL-6, IL-1β, *S*-adenosyl-homocysteine, and soluble CD8, IL-2 receptor, and intercellular adhesion molecule-1 are increased in CSF during HIV infection. However, increases in these factors have either been subjected to limited investigation or have been correlated more with infection, stage of infection, or blood-brain barrier dysfunction than with neurological disease, so their links to dementia are unclear. CSF factors that are indicative of HIV-associated dementia when elevated are: neopterin, β_2-microglobulin, prostaglandin E2, quinolinic acid, platelet-activating factor, and monocyte chemotactic protein-1 (MCP-1) (27–31). All are products of activated macrophages, which further highlights the role of macrophages in the pathogenesis of HIV-associated dementia. Levels of these factors in CSF frequently rise concomitantly as dementia worsens, suggesting that they are produced in response to, or contribute to, the same local CNS conditions (Fig. 3). Some of these products may be directly cytotoxic for neurons or induce neuronal dysfunction. For instance, quinolinic acid and platelet-activating factor are neuronal excitotoxins (31, 32).

In general, the most important mechanism for induction of macrophage activation is T cell activation, particularly production of IFN-γ. Although levels of IFN-γ mRNA do not differ between demented and nondemented individuals (Fig. 1), it is possible that the lack of IL-4 counteractivity in demented patients results in high effective levels of IFN-γ in these patients (Fig. 2).

Conclusions

Uncontrolled immune responses in the nervous system are potentially damaging. Comparison of detrimental and nondetrimental responses to virus infections of the brain and spinal cord suggest that type 2 cytokine responses that favor local

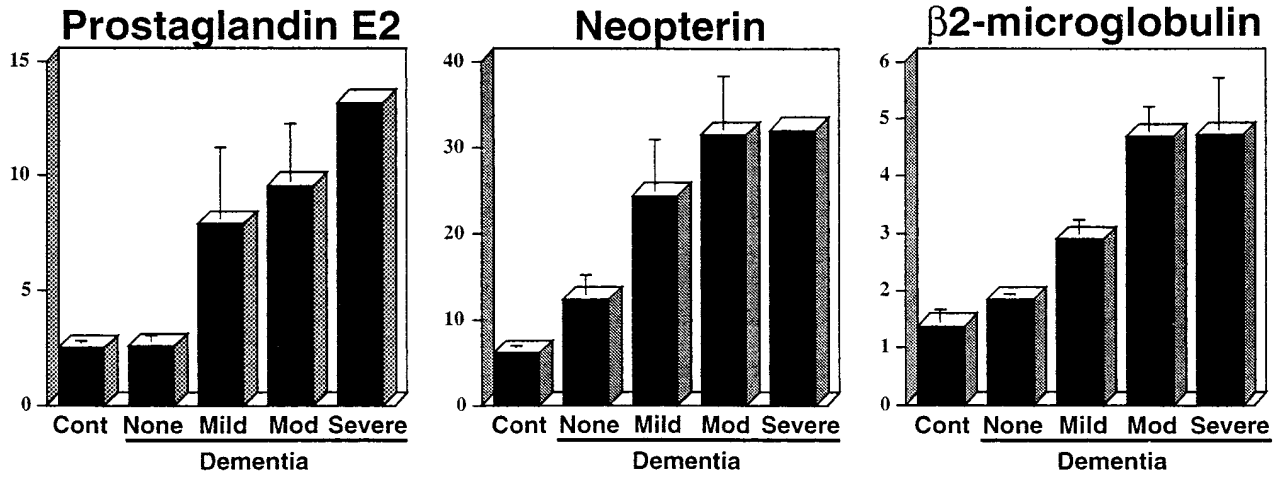

Figure 3. Products of activated macrophages in the CSF of HIV-infected individuals with and without dementia. Levels correlate with the severity of dementia. Reproduced with permission from Wesselingh, Tyor, and Griffin, *In* Cytokines and the CNS, CRC Press, 1996.

antibody production rather than macrophage activation are desirable. The CNS possesses a number of intrinsic mechanisms for controlling local immune responses including the blood-brain barrier, high levels of gangliosides, and controlled expression of MHC antigens and local cytokines. HIV-associated dementia is characterized by increased numbers of activated macrophages producing a variety of products that may be directly or indirectly toxic to neurons. It is hypothesized that the defining abnormality for HIV-infected individuals who develop dementia is failure to control the activation of macrophages within the CNS due to insufficient production of downregulating cytokines.

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