Commentary

Cerebral Endothelial Activation and Signal Transduction Mechanisms during Inflammation and Infectious Disease

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Induction of cell surface molecules on cerebral endothelial cells, microglia, oligodendrocytes, choroidal epithelial cells, and (to a lesser degree) astrocytes has been reported in a few chronic inflammatory conditions and infectious diseases of the central nervous system (CNS). The elucidation of autoimmune mechanisms in multiple sclerosis and its experimental model, experimental allergic encephalomyelitis (EAE), has provided knowledge of the pleiotropic actions of cytokines and specific cellular interactions between the circulation-derived immune cells and brain elements. Although stereotypical immune interactions are mimicked in the CNS, they do not appear to measure up to what is encountered within systemic organs. This is due not only to the specialized cellular consistency of the CNS but also to its isolation behind the blood-brain barrier. It may not, therefore, be surprising that the targets, namely endothelial cells or macrophages (microglia) of the brain, do not fully respond to injury or infection in the same manner as or with the expected resilience of those in the periphery. However, inflammatory responses resulting from infections or injury of the CNS activate the brain endothelium and other nonneural cells of the brain to various degrees depending on the type, titer, or strength and duration of exposure to the agent or insult. The activation of these cells may be modulated by the action of one or more cytokines and relies on expression of respective cell surface receptors, though cytokines are known to work via receptor-independent systems. In an effort to understand the immunopathogenesis and find rational treatments several rodent and nonhuman primate models of infectious disease of the CNS have been devised. Such efforts have further been rewarded by the use of several mouse knockout strains or models deficient in molecules of interest.

Infections of the CNS by viruses, bacteria, and protozoa reveal the presence of inflammatory nodules and perivascular cuffing around blood vessels by inflamma-

tory cells including infiltrated lymphocytes, monocytes, and microglia. Astrocytes and oligodendrocytes also invariably become hypertrophic and exhibit hyperplasia. However, the consequences of these agents on neurons remain largely unknown. Protozoal infections of the CNS such as cerebral malaria (*Plasmodium falciparum*), toxoplasmosis (*Toxoplasma gondii*), and trypanosomiasis (*Trypanosoma brucei* and Chagas' disease) lead to unique immunopathological features, although they also have features in common with those apparent in chronic inflammatory conditions such as multiple sclerosis. The molecular mechanisms involved in these conditions and how these might impinge on understanding brain inflammatory mechanisms are largely unexplored topics. Toxoplasmosis, an obligate intracellular protozoan, has recently attracted considerable attention because it is the most common CNS infection producing a mass lesion in acquired immune deficiency syndrome (AIDS). Ten to 25 percent of AIDS patients have toxoplasmosis of the CNS and *Toxoplasma* abscesses are a late complication of HIV infection associated with a low CD4⁺ cell count. It is well known that during chronic infection in AIDS and mouse models of toxoplasmosis, parasite-specific T lymphocytes release high levels of the cytokine interferon- γ (IFN- γ), which is required to prevent cyst reactivation and likely initiates several cascades of inflammatory and immune responses.

Specific Actions of Cytokines on Cerebral Endothelium and Microglia

In vitro and *in vivo* studies demonstrate that cytokines such as IFN- γ and tumor necrosis factor- α (TNF- α) can readily induce a variety of critical cell adhesion molecules as well as the major histocompatibility complex (MHC) antigens in nonneural cells of the CNS. IFN- γ and TNF- α seem equally important stimulators of endothelial cells,

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microglia and oligodendrocytes but not astrocytes or neurones. Both class I and class II MHC molecules may be readily induced in brains of rats and mice depending on the dose and duration of the presence of IFN- γ .^{1,2} It is now well recognized that the induction of these molecules or adhesion proteins, such as intracellular cell adhesion molecule (ICAM-1), in endothelial cells as well as microglia depends also on the strain and species of animal. For example, IFN- γ or TNF- α can elicit more robust responses in mice susceptible to EAE or cerebral malaria than in disease-resistant mouse strains.³ IFN- γ induced expression of MHC class II was significantly higher in mice susceptible to cerebral malaria than in resistant mice. Similarly, site-specific responses have been noted with respect to these cytokines. Inasmuch as there appear to be certain differences in the induction of MHC antigens in endothelial cells between cerebral and peripheral vessels, it is known that circulation-derived macrophages display a more robust antigen-presenting capacity function than brain-resident microglia.⁴ The activation stage of cells is another variable important to achieving the desired outcome. For example, the antigen-presenting capacity of activated (but not unactivated) microglia to naïve T cells could be increased by treatment with IFN- γ .⁵

The differential actions and the modulation of responses of the cytokines may be achieved via different receptors. Deckert-Schluter and colleagues⁶ previously showed that expression of inducible nitric oxide synthase (iNOS) was necessary by signaling through the 55-kd TNF receptor-1 (TNFR1) rather than the 75-kd TNFR2. In mice subjected to encephalitis by *Toxoplasma gondii* infection the expression of iNOS protein and mRNA in microglia were reduced, whereas that of IFN- γ , TNF- α , or interleukin-1 β (IL-1 β), as well as the recruitment of immune cells in brain, were unaffected in TNFR1-deficient mice. Whether such preferential signaling occurs in the cerebral endothelium is unknown. In accord with the differential effects of TNFR, however, tissue factor synthesis was regulated through TNFR1 but not TNFR2. This study also showed that endothelial secretion of tissue factor occurs via the synergistic action of TNF and vascular endothelial growth factor.⁷

In this issue of *The American Journal of Pathology*, Deckert-Schluter et al⁸ report on the role of IFN- γ R- and TNFR-mediated immune reactions in the activation of cerebral endothelial cells in the same mouse model of *Toxoplasma* encephalitis. Although a strong up-regulation of the cell adhesion molecules vascular cell adhesion molecule (VCAM) and ICAM-1 and the MHC antigens was observed in wild-type and TNFR-deficient mice with *Toxoplasma* encephalitis, it was not observed in the IFN- γ R-deficient mice. Similar lack of up-regulation of ICAM-1 and its ligand LFA-1 and of the MHC class I and II molecules was also noted in microglia of the IFN- γ R-deficient mice compared to the TNFR-deficient or wild-type mice. These observations suggest that the IFN- γ R rather than the TNFR signaling pathway is involved in activation of the cerebral endothelium and microglia in mice with *Toxoplasma* encephalitis. Whether such signaling is specific to the condition remains to be seen but it is

not unlikely that there will be differences, albeit small ones, between various inflammatory conditions and infectious diseases, including cerebral malaria. However, the study also showed that although maximal induction of VCAM and ICAM was IFN- γ -dependent, the IFN- γ R-deficient mice showed significant up-regulation of VCAM upon infection. This observation suggests that, in addition to IFN- γ , other factors such as IL-1 β (also produced in IFN-deficient mice) may be involved in the up-regulation of these molecules. That IFN- γ -independent factors are important in other CNS infections is evident in another mouse encephalitis caused by the lymphocytic choriomeningitis virus, where normal induction of ICAM-1 and VCAM was observed in IFN- γ -deficient mice.⁸ These findings suggest that there may be considerable downstream cross-talk between different signaling pathways in response to particular pathogenetic mechanisms.

Deckert-Schluter and colleagues further demonstrated that the recruitment of immune cells (CD4+, CD8+, and macrophages) was not impaired in IFN- γ -deficient mice and was similar to that in wild-type mice.⁸ They suggest that the low level of induction of cell adhesion molecules was sufficient in IFN- γ -deficient mice to ensure entry of leukocytes into CNS. However, recruitment of macrophages but not leukocytes was impaired in the TNFR-deficient mice. This is comparable to the EAE mouse model where TNF- α deficient mice also had impaired recruitment of macrophages but not of T cells to the brain.⁹ These observations imply that some cytokines may play divergent roles in guiding the movement of inflammatory cells into brain parenchyma that may be explained by different pathogenic mechanisms of such diseases.

Synergistic Actions of Cytokines

Recent studies with brain endothelial cultures have shown that IFN- γ and TNF- α also act in synergy with each other or with IL-1 but it is unclear yet how they might interact and differentially regulate downstream signaling pathways. Tanaka and McCarron¹⁰ had previously shown that TNF- α inhibits induction of Ia antigens by IFN- γ in cultures of cerebral endothelial cells and that IL-1 may act synergistically with TNF- α to down-regulate or alter immune responses in the endothelial cells. Using primary cultures of human brain endothelial cells, Dorovini-Zis and colleagues¹¹ have clearly shown that unlike TNF- α , IFN- γ has little or no effect in inducing cell adhesion molecules including ICAM-1, VCAM-1, and E selectin. However, IFN- γ and TNF- α in combination maximally induced ICAM-1. In contrast, IFN- γ alone readily induced MHC class II antigens in the endothelial cells and likely increased permeability of the endothelium via receptor-independent mechanisms. Interestingly, even in bovine brain endothelial cells¹² endothelin-1 (and probably other vasoactive substances) was induced by the combined actions of TNF- α and IFN- γ . Therefore, direct actions of cytokines that may result in a change in tone or permeability of the cerebrovascular endothelium should not be overlooked.

Activation of iNOS and production of nitric oxide (NO) further illustrate the synergistic actions of cytokines. The combination of IFN- γ and TNF- α (with or without IL-1 β) was required to induce iNOS and its mRNA in brain endothelial cells and in microglia from rats and mice. Such induction occurs via protein tyrosine kinase and the transcription factor NF- κ B. IFN- γ , in concert with TNF- α , also markedly enhances the ability of IL-1 β -primed endothelial cells to release reactive nitrogen intermediates, which may affect vascular tone and permeability. The synergistic actions of cytokines such as IFN and TNF are undoubtedly important in magnifying their biological effects during brain injury and inflammation. For example, IFN- γ can induce TNFR1 and TNFR1 mRNA in cerebral endothelial cells from EAE-susceptible mice.¹³ In support of this, Deckert-Schluter et al⁸ also demonstrated that TNF mRNA was not induced in the IFN- γ -deficient mice with *Toxoplasma* encephalitis. A recent study suggesting that induction of TNFR1 by IFN- γ facilitates the actions of TNF- α at the transcription level shows the importance of this finding in oligodendrocytes,¹⁴ although it is not unlikely that brain endothelial cells may respond in a similar manner. Agresti et al¹⁴ demonstrated that neither IFN- γ nor TNF- α was capable of inducing MHC class I regulatory element (MHC-CRE) binding activity when administered alone. However, following exposure of rat oligo-dendrocytes to IFN- γ , TNFR1 expression was transcriptionally induced by the binding of signal transducer and activator of transcription-1 (STAT-1) homodimers to the IFN- γ -activated site (GAS) present in the gene promoter. The up-regulation of TNFR1 allowed TNF- α to induce binding of NF- κ B to the MHC-CRE site. Thus, IFN- γ and TNF- α synergistically stimulated interferon regulatory factor (IRF-1) gene expression. IFN- γ directly induced the binding of STAT-1 homodimers to the GAS element, whereas NF- κ B binding to κ B sequence was activated by TNF- α only after IFN- γ treatment.

IFN- γ R and TNFR- α Signaling

Current studies show convincingly that the actions of TNF in endothelial cells are mediated via protein tyrosine kinase and protein kinase C (PKC), rather than protein kinase A (PKA), signaling pathways.^{15,16} Human microvascular endothelial cells exposed to ultraviolet light B irradiation induced ICAM-1 up-regulation and lymphocyte-endothelium interaction.¹⁷ These authors also observed that PKC inhibitors rather than PKA inhibitors were effective in attenuating this induction and that PKC- α was translocated from the cytosol to the membrane, indicating enzyme activation. However, IFN- γ but not TNF- α antibodies blocked the irradiation-induced ICAM-1 up-regulation. In another approach, Grau et al³ described TNF- α -induced up-regulation of both TNFR1 and TNFR2 receptors in brain endothelial cells from cerebral malaria-susceptible but not from resistant mice. PKC inhibitors blocked the response to TNF- α in both mouse strains but an inhibitor of PKA selectively abolished the response to TNF- α in the cerebral malaria-resistant mice. Although

these observations support the importance of TNF-mediated PKC-dependent signaling in certain inflammatory conditions, they collectively imply divergent signaling pathways for cytokine-induced adhesion molecule expression.

IFN- γ and several other cytokines have been found to activate the Janus kinases (Jaks) and the STAT proteins.¹⁸ Although similar signaling ought to be present in all cells expected to respond to cytokines, the components of the signaling pathways have not yet been characterized in CNS cells with IFN- γ R or other cytokine receptors. Jaks are a unique class of tyrosine kinases that associate with cytokine receptors. On ligand binding, they activate (see above) members of the STAT family through phosphorylation on a single tyrosine. Activated STATs form dimers, translocate to the nucleus, bind to specific response elements (eg, GAS) in promoters of target gene, and transcriptionally activate these genes. Both positive and negative regulations of the Jak-STAT pathway have been identified. It has also been shown that STAT-1 knockout mice have impaired IFN signaling.¹⁸

Convergence of the actions of TNF- α and IFN- γ is thought to occur through the interferon regulatory factor (IRF-1). The induction is a primary transcriptional response that occurs rapidly without further requirement for protein synthesis. Synergism is mediated by a novel composite element in the IRF-1 promoter that includes GAS, overlapped by a nonconsensus site for NF- κ B. Synergistic induction of IRF-1 is likely to be an important early step in regulatory networks critical to the synergism of TNF- α and IFN- γ . The GAS/NF- κ B element may mediate synergistic transcriptional induction of IRF-1 by other pairs of ligands that together activate NF- κ B and STAT family members. There is also some evidence to suggest that transmodulation between the STAT and SMAD (homologues of *Drosophila* mao proteins) signal transduction pathways occurs, providing a further means of cross-talk between IFN- γ and TGF- β signaling pathways.

Cerebral endothelial cells and microglia are activated by cytokines to induce a variety of cell surface molecules during inflammation and infectious disease. The degree of induction may depend not only on the nature of the cytokine but also on the pathogenetic features of the condition.¹⁹ Although IFN- γ and TNF- α act independently, it is apparent that their synergy in the induction of characteristic cell adhesion and MHC molecules is important in modulation of specific responses in certain CNS infections. However, the specific downstream signaling components after cytokine receptor stimulation remain to be elucidated for a full understanding of immune responses in models of infectious disease of the CNS such as *Toxoplasma* encephalitis and cerebral malaria. Although more peculiarities of cytokine actions remain to be elucidated in the pathogenesis of these models or other CNS diseases, it is important to bear in mind that variable results often arise due to different experimental designs, time point(s) of the course of infection being studied, and species and strains of animals used.

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