Journal of NeuroVirology (1999) 5, 533–537

http://www.jneurovirol.com

Guest Editorial

The blood-brain barrier: a defensive shield or a perpetrator of microbial invasion?

We are pleased to publish, in this special issue of the *Journal of NeuroVirology*, a series of articles that highlight the blood-brain barrier's (BBB) importance in brain defense and microbial pathogenesis. The multifaceted role of the barrier as an immune competent organ, as a protective barrier and as a regulator of blood to brain transport of vital nutrients, proteins, vitamins and electrolytes cannot be overstated. Knowledge of BBB function is certainly a central part of the field of Neurovirology. Thus, it is our intent to provide the reader with a broad range of information for each of these functions in the context of microbial neuropathogenesis. The works are divided into four parts in order to accommodate the plethora of functions and microbes that affect BBB biology. The sections include reviews of the functional biology and immunology of the BBB; the diverse roles played by the barrier in response to microbial infection; and the emerging role that chemokines and their receptors play in leukocyte trafficking into the central nervous system (CNS), as receptors for viral infection, and as perpetrators for disease. Although HIV/SIV infections are a focal point of the *Journal's* special issue on BBB function, the diversity of topics and pathogens covered provide a broad review of the field. The mechanisms of viral entry into the brain, the role that BBB immunity plays in leukocyte trafficking, and the influence of peripheral viral and cellular responses in altering barrier integrity are all highlighted in this monograph.

The functional biology and immunology of the blood-brain barrier (BBB)

A major, but not exclusive, function of the BBB is to regulate the passage of immune cells, proteins, and other nutrients from the blood to the brain. This function serves to protect the nervous system against circulating pathogens and toxins that would disrupt brain function. This barrier composed of brain microvascular endothelial cells, astrocyte foot processes and capillary pericytes is also immunologically active. The continuous interplay between the functional biology and immunology of the BBB makes it a unique organ that can serve both as a protector against and facilitator of microbial invasion. Thus, the BBB can clearly effect microbial-induced neurological disease. During steady state conditions, the BBB is relatively impermeable to proteins due to its tight junctions and low transcellular flux. Only rarely do leukocytes migrate across the BBB in health. It is during inflammation that activated monocytes and lymphocytes readily cross the barrier and do so in abundance. Structural changes in the barrier or subtle metabolic disturbances in its function may, in part, mediate such inflammatory cell migrations. A greater number of monocytes than lymphocytes can enter the CNS perivascular space. Infiltration of these cells is controlled, in part, by the state of brain endothelial cell activation and also through interaction with inflammatory cell factors which control cell adhesion and ultimately leukocyte transendothelial migration. Inflammation (when it occurs in brain during disease) is associated with an upregulation of class I and class II antigens, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM), and interleukin one (IL-1), as well as altered endothelial cell morphology. These factors result in increased binding of inflammatory cells, permitting their passage into the brain parenchyma. Leukocyte migration from blood to brain is a complex process involving rolling, adhesion, trans- and subendothelial migration. These steps are mediated by adhesion molecules and their ligands and include: (a) selectins, proteins with calcium-dependent lectin-like binding domains, (b) ligands for selectins, which include sialylated, fucosylated and sulfated carbohydrates and other membranes of the sialomucin family (glycosylation-dependent cell adhesion molecule-1, and CD34), (c) endothelial-like proteins and (d) leukocye 1 and 2 integrins, which include CD11a/CD18 (lymphocyte function associated antigen-1, LFA-1) present on lymphocytes and LFA-1, Mac-1, p150 and p95 present in neutrophils, monocytes and NK cells. Chemoattractive cytokines (chemokines) also expressed by monocytes/macrophages and lymphocytes play an important role in activating integrin adhesiveness and directing migration of leukocytes. Thus, the capillaries that form the BBB contain a diverse functional array of molecules that allow passage of cells, virus, and other microbial pathogens between blood and brain (Banks, 1999;

Pardridge, 1999; Miller, 1999; Persidsky, 1999). In our attempt to provide the reader with a broad understanding of such complex BBB biology, the contributors were selected based on their ability to address basic features of the barrier in homeostasis and in disease. The first section addresses the restrictive properties of the BBB emphasizing the role of the cell monolayer separating the periphery from the brain. Indeed this physiologic separation contains complex control mechanisms necessary for regulating the nervous systems' environment rather than just a physical blockade (Banks, 1999). The overall brain microvascular biology involves and relies on paracrine interactions between brain microvascular endothelium, the capillary pericyte, and the astrocyte and its foot processes (Pardridge, 1999; Miller 1999). The BBB is both a target site for immune regulatory responses (including effector cell secretory activities) and a conduit for activation of both innate and acquired immune responses within the CNS. It is these very responses that permit the BBB to regulate leukocyte entry into the brain during microbial infection or to act as perpetrators of the disease process.

Microbial pathogenesis and the blood-brain barrier

In order to link BBB dysfunction with microbial neuropathogenesis, a second section for this special issue was developed that reviews disease variability with regard to pathogens that infect the CNS. Viruses may invade the brain, infecting neurons and/or glia, and elicit an immune reaction in the CNS parenchyma producing meningitis, encephalitis, or both. They may enter the brain within leukocytes crossing the BBB, or through direct infection of brain microvascular endothelial cells. Bacteria, on the other hand, enter the cerebrospinal fluid (CSF) compartment and cause meningitis. Zhang and Tuomanen (1999) review the capabilities that enable diverse pathogens to enter the brain and cause distinct disease processes. Once inside the brain, viruses can cause neuronal injury. Such injury is dependent upon a number of factors, including viral cell tropism, glial immune regulatory events and regional pathology. For example, insights into normal brain function can be gleaned from studies addressing the impact of neurotropic viruses and/or CNS immune factors on complex neural functions including behavior and movement. Studies of human Borna disease virus (BDV) infection provides unique insights into mechanisms of viral neuropathogenesis, as well as advances in our understanding of neuropsychiatric disorders (Briese *et al*, 1999). Theiler's murine encephalomyelitis virus (TMEV) induces a demyelinating disease involving immune reactions acquired inside the brain. Here cellular immune responses directed initially to infected macrophages and oligodendrocytes causes white matter destruction by an autoimmune mechanism. $CD4^+$ T lymphocytes are believed to mediate this CNS pathology. Viral antigen-specific T lymphocyte proliferation and delayed type hypersensitivity (DTH) responses are detected early in the course of viral infection. Later epitope spreading occurs. This is characterized by the evolution of viral cellular immune reactions to autoimmunity. Antigen-specific T lymphocyte proliferation and DTH responses to host proteolipid peptides continue to occur 50 days after viral infection (Hoffman *et al*, 1999). For measles virus infection CNS complications can occur immediately following viral infection or years after exposure. Acute postinfectious measles encephalitis, measles inclusion body encephalitis (in immunocompromised patients), and subacute sclerosing panencephalitis are all manifestations of viral infection but unique pathologies. The various clinical and pathological manifestations of viral infection in brain reflect the route of invasion, the immune responses, the viral strain(s) and genetic susceptibilities of the host (Schneider-Schaulies *et al*, 1999).

Chemokines, blood-brain barrier function and CNS viral invasion

A rapidly emerging area in studies of leukocyte migration, disease pathogenesis, and viral infection are chemokines and their receptors. These chemoattractant molecules are tightly linked to CNS disease through their ability to play pivotal roles in a variety of microbial infections, immune responses, and degenerative events within the nervous system. In the first paper of this series, Drs Glabinski and Ransohoff provide an overview of chemokines and their receptors in CNS homeostasis and disease. Since the primary triggering elements in the initiation of CNS inflammation are antigen-specific T cells and/or monocyte-derived macrophages, the mechanisms by which these cells are recruited into the brain is of prime importance for disease. After penetration of the BBB, blood-based immune effector cells stimulate microglial, endothelial and astrocyte chemokine production. Chemokines regulate migration of leukocytes from the circulation into the CNS and their composition and mode of regulation play central roles in inflammatory disease (Glabinski and Ransohoff, 1999). In CNS autoimmune diseases (for example, multiple sclerosis and experimental autoimmune encephalomyelitis), chemokines directly participate in the disease process. This is also evident for microbial infections of the brain. In TMEV the development of clinical disease is strongly associated with chemokine expression in the CNS. Which chemokines and at what levels they are produced dictate the tempo of

demyelination, a critical component of TMEV pathogenesis (Hoffman *et al*, 1999). For HIV-1 and SIV infections of the CNS, several members of the chemokine receptor family are used as coreceptors for viral infection along with CD4. CCR5 is a major coreceptor for HIV-1 infection of mononuclear phagocytes (MP) (brain and perivascular macrophages and microglia). CXCR4 and CCR3 may mediate infection in other neural cell types but at reduced efficiency. Recent works suggest that such receptors, notably CXCR4, can also affect mechanisms of neural injury. In HIV-1 associated dementia (HAD), MCP-1, strongly influences monocyte-derived macrophage migration into the brain and thus creates an expanding viral reservoir as well as sites of brain immunity against virus. Certainly, chemokine receptors and their ligands can affect viral tropism and mechanisms of CNS pathology in lentivirus-associated encephalopathies (Gabuzda and Wang, 1999). Defining their role in disease will likely be a central pursuit for research efforts much into the next decade.

Regulation of BBB function in HIV-1/SIV infections: role of viral/cellular proteins in leukocyte migration and in disease

This section serves to bring into focus the articles that proceed it. Indeed, the BBB may influence HIV-1 disease in a variety of ways. First, direct infection of brain microvascular endothelial cells may act as a conduit for viral infection from the peripheral circulation to the brain (Moses *et al*, 1993). Second, peripheral and/or CNS immune responses may lead to cytokine dysregulation with concomitant alterations in BBB integrity, disruption of tight junctions, and adhesion of leukocytes to the vascular endothelium. These events, taken together, lead to inflammatory cell transendothelial migration into the brain and the perpetration of the disease process. When virus invades the CNS it can initiate an immune activation response. This results in chemokine secretion and a cascade of regulatory events that affect monocyte migration into the CNS. In turn, such events serve to expand the reservoir for virus in brain and provide a source of immune effector molecules with neurotoxic potential. These issues are discussed, in whole or part in the articles that follow.

Clearly, the infiltration of blood-borne monocyte-derived macrophages into the brain characterizes the clinical and pathological manifestations of HAD. Secretory products from virus-infected brain macrophages/microglia directly affect CNS damage and BBB permeability (Persidsky, 1999; Nottet, 1999). Virus-induced brain pathology includes neuronal loss, astrocytosis, myelin pallor, and a variety of inflammatory cell responses. It is the immune secretory products, which include eicosanoids, pro-inflammatory cytokines and chemokines, that most affect BBB function and lead to attraction of monocytes into brain. Neuropathological findings, including perturbations of the BBB, are strongly associated with viral infection and activation of brain mononuclear phagocytes (MP). The recruitment of MPs, specifically blood monocytes, into brain governs the tempo and progression of CNS disease (Nottet, 1999). The mechanisms responsible for monocyte entry into brain during HIV infection involve both peripheral and CNS immune responses. This has important consequences for therapy and for understanding mechanisms of disease pathogenesis. Indeed, preventing monocyte recruitment into the CNS could ultimately abrogate neurotoxin production and thus influence the course of neuronal injury (Persidsky, 1999).

Several studies demonstrate viral entry into the CNS either during the acute seroconversion reaction or at times of clinical infection. Nonetheless, neurological manifestations of cognitive and motor impairments usually occur late in the course of disease. Clinical events often begin during advanced immunosuppression with an associated high-level plasma and CSF viremia. Reseeding of the brain by HIV late in the course of disease through a pronounced infiltration of MP, likely heralds an expansion in viral CNS load and brain immunological activation and the onset of neurological disease. The number of MPs in brain parenchyma is a much better correlate of HAD than the presence, location, or concentration of virus in the brain. These observations, taken together, suggest that HIV infection is necessary, but not sufficient, to produce neurological disease. Both HIV-1 infection and activation of brain macrophages/microglia, mediated by the brain's inherent regulatory factors or by secondary viral, bacterial, fungal and/or parasitic infections, are necessary for neural injury. In support of this idea, large numbers of brain macrophages/microglia expressing MHC Class II, IL-1, and TNF antigens are readily found in brain during advanced clinical disease (Nottet, 1999). Together, these factors induce the expression of adhesion molecules on brain microvascular endothelial cells and thus influence the transendothelial migration of monocytes. HIV might gain entry into the brain tissue through either a Trojan horse mechanism within infected monocytes, through direct infection of endothelial cells, by infection of the choroid plexus, or through infected T cells. The choroid plexus, often overlooked in past works, is an important site for viral dissemination in the brain. This is shown by the fact that monocytes and dendritic cells in the stroma of the plexus are infected at considerably high levels. The study by Petito *et al*, 1999 shows that the choroid plexus is a critical site for HIV infection in the brain. In

either of these scenarios HIV, its products, or secretory factors from immune competent monocytes would induce the expression of adhesion molecules on endothelial cells or similar secretions might directly damage the integrity of the BBB and the vascular endothelium. For example, HIV-1 Tat has been clearly implicated in CNS viral pathogenesis and BBB perturbations. Tat induces a dysregulation of both cytokines and chemokines and may play an important role in the trafficking of inflammatory cells, particularly monocytes, through the barrier. HIV-1 Tat protein is found in microglia and astrocytes in HAD as well as in Progressive Multifocal Leukoencephalopathy (PML). The ability of Tat to activate chemokine expression is consistent with the activation of MIP-1α in these lesions. Thus, the ability of HIV-1 infection, *per se*, or specifically its regulatory protein, Tat, to induce chemokines may affect monocyte/macrophage invasion into the brain during HAD (Bonwetsch *et al*, 1999). This may occur concomitantly with immune activation. Since activated HIV-infected monocytes overexpress pro-inflammatory cytokines, for example TNF also potentially activated by tat, the activated cells may have a selective advantage in transendothelial migration. The disruption of the BBB ultimately permits entry of monocytes into the CNS parenchyma. The perivascular leukocytes in and around the macrovasculature, and proliferation of astrocytes all enhance such monocyte transendothelial migration. The relative roles of LFA-1/ICAM-1 and VLA-4/VCAM-1, receptor-ligand pairs may be altered according to the state of activation in brain. Current experimental evidence indicates that the initial step in this adhesion process is mediated by the selectins, L-selectin, E-selectin and P-selectin. Firm adherence to endothelial cells, however, requires activation and engagement of the integrins, for example ICAM-1 and ICAM-2. The functional competence of integrin-dependent adherence requires monocyte stimulation by chemokines or perhaps PKC activation. These stimuli induce qualitative changes in intracellular signaling. Endothelial cells may also play an active role in augmenting leukocyte adhesion. For example, stimulated endothelial cells produce the lipid mediator platelet activating factor, which is also produced in abundance by immune-activated HIV-1-infected monocytes. A complex but integrated array of molecules control attraction, adherence, and transendothelial migration of monocytes into the brain (Persidsky, 1999). Interestingly, the expression of ICAM and other integrins are markedly upregulated when the endothelium is exposed to TNFα, IL-1, or interferon-gamma (IFNγ).

The special features of the endothelium include the presence of tight junctions and alterations in glucose transport. Glucose transporter proteins in the brains of SIV-infected macaques decline significantly in animals with moderate to severe encephalitis. Such alterations could reflect significant disturbances in the CNS microenvironment and contribute to BBB dysfunction (Mankowski *et al*, 1999). Brain endothelial cells may be impregnable to most macromolecules and prevent entrance of circulating leukocytes into the brain.

Astrocytes are a structural and functional component of the BBB (Woodman *et al*, 1999). Astrocyte foot processes are found in close opposition to the abluminal surface of the brain endothelium and participate in BBB formation. The idea that astrocytes induce alterations in the BBB is now generally agreed upon. For example, HIV-1 Tat induces adhesion molecule and chemokine expression in astrocytes. Thus, the complexities of the BBB structure and function, coupled with alterations in virus and neuro-immune activation during advanced HIV disease, present the principle mediators for the regulation of monocyte entry into brain. Future studies of BBB function should and will permit the unraveling of the cellular and molecular events involved in the transendothelial migration of monocytes into brain and the subsequent development of neurological diseases induced by viral infection of the CNS.

Therefore and in summary, we have included a variety of articles on the BBB that encompass the biology and functional immunology of this most important CNS shield. However, even considering the huge progress that has been made in understanding barrier function in health and disease, we collectively have a way to go to fully comprehend the contributions of this organ for brain homeostasis. It is hoped that this special issue will serve as an impetus for future research in this area and that important contributions will continue leading to therapeutic advances for neurovirological diseases.

Acknowledgements

The editors wish to express their sincere appreciation to June Vieth (Editorial Assistant, *Journal of NeuroVirology*) and Robin Taylor (University of Nebraska Medical Center) for editorial, logistical and organizational support for this special issue. Their hard work and attention to detail was responsible, in large measure, for the timeliness of this monograph. The authors need to be congratulated for their excellent contributions and stimulating discussions that turned an idea for a special issue on the BBB into a reality. A special thanks is offered to Drs James Linder and Samuel Cohen (University of Nebraska Medical Center) for providing the continuous support and inspiration necessary to complete this endeavor.

Lastly, we wish to thank Dr Kamel Khalili, Editor-in-Chief, whose inspiring vision for the *Journal of NeuroVirology* made this issue and countless other pursuits possible.

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