Journal of NeuroVirology (1999) 5, 579–590

http://www.jneurovirol.com

Review

Model systems for studies of leukocyte migration across the blood–brain barrier

Yuri Persidsky*,[1]

[1]*Department of Pathology and Microbiology, Center for Neurovirology and Neurodegenerative Disorders, 985215 Nebraska Medical Center, Omaha, Nebraska, NE 68198-5215 USA*

The blood–brain barrier (BBB) plays a crucial role in central nervous system (CNS) homeostasis. Serving as the brain's protective shield it regulates soluble factor and cellular exchanges from blood to brain. Critical to its function, the BBB is composed of brain microvascular endothelial cells (BMVEC), a collagen matrix, and astrocytes. Astrocytic endfeet surround the BMVEC abluminal surface and influence the 'tightness' and trafficking role of the barrier. In neurodegenerative disorders (for example stroke, multiple sclerosis and HIV encephalitis) the BBB becomes compromised. This is, in part, immune mediated. An accumulating body of evidence demonstrates that the cellular components of the BBB are themselves immunocompetent. Perivascular cells (astrocytes, macrophages and microglial cells) and BMVEC produce inflammatory factors that affect BBB permeability and expression of adhesion molecules. These affect cell trafficking into the CNS. Leukocyte BBB migration can be influenced by cytokines and chemokines produced by glia. Astrocytes and macrophages secrete a multitude of factors that affect brain immune responses. Interactions between BMVEC, leukocytes and/or glia, immunological activation and noxious (infectious, toxic and immune-mediated) brain insults all appear to play important roles in this BBB cell trafficking. New information gained into the mechanisms of leukocyte-brain penetration may provide novel insights in the pathogenesis and treatment strategies of neurodegenerative disorders.

Keywords: blood–brain barrier; leukocyte migration; models; neuroinflammatory products

Introduction

Leukocyte transendothelial migration across an altered blood–brain barrier (BBB) is a prominent feature of many neurodegenerative disorders. Nevertheless, the events that control entry of blood cells into brain, affecting the progression of neurological diseases, have not been fully elucidated. To this end, this article reviews BBB structure and function during normal brain homeostasis and during disease. The functions of BBB include separation of the brain from activities in the periphery, selective transport of factors necessary for CNS homeostasis, and metabolism of blood- or brain-borne macromolecules (Risau and Woburg, 1990). Under normal conditions, the BBB efficiently restricts movement of ions, proteins and other polar organic molecules to the brain. This is regulated, in part, by the BBB's high electrical resistance (Pardridge, 1983). Structurally, the BBB is composed of specialized non-fenestrated microvascular endothelial cells (BMVEC) connected in a impermeable monolayer by tight junctions devoid of transcellular pores (Pardridge, 1983). Additional components of the barrier are the surrounding capillary basement membrane and astrocytes. Astrocyte end-feet are in close apposition to the abluminal surface of the brain endothelium and assist in the barrier function by coordinating the functional activities of BMVEC (Janzer and Raff, 1987; Meyer *et al*, 1991; Tontsch and Bauer, 1991).

*Correspondence: Y Persidsky, Center for Neurovirology and Neurodegenerative Disorders, 985215 Nebraska Medical Center, Omaha, Nebraska, NE 68198-5215, USA
Received 29 June 1999; revised 4 August 1999; accepted 6 August 1999

The structural and immune integrity of the BBB is highlighted by its limited major histocompatibility complex (MHC) antigen expression, its diminished antigen-presenting function and increased threshold for immune activation. These collectively contribute to the 'immunological privilege' of the CNS. Under normal homeostatic conditions leukocyte brain infiltration is highly restricted (Carson and Sutcliffe, 1999; Nottet and Dhawan, 1997).

Transendothelial migration of cells into the brain is regulated by sequential immune events involving endothelial signaling molecules (Hickey, 1997). In 1991, Butcher (1991) proposed that leukocyte-endothelial interactions involve an active multistep process. The first step, rolling of leukocytes over an endothelium, is mediated by the selectin family of molecules (C-type lectins). This includes the endothelial antigens E-selectin and P-selectin and their ligands (surface glycoproteins) present on leukocytes. Firm adhesion of leukocytes to the endothelial surface is regulated by 'integrins'. These are heterodimeric proteins which mediate cell-cell and cell-extracellular matrix adhesive connections [for example, macrophage-1 antigen (Mac-1), very late activation antigen 4 (VLA-4), and lymphocyte function-associated antigen 1 (LFA-1), LFA-2, LFA-3]. Integrins bind to their corresponding adhesion molecules expressed on activated endothelial cells [for example, VLA-4 binds to vascular cell adhesion molecule 1 (VCAM-1) and Mac-1 and LFAs attach to intercellular adhesion molecules (ICAMs)]. The second step involves chemoattractants present on the vessel wall or in the near vicinity of the vessel. This process is independent from the original rolling and tethering of leukocytes and the endothelium. This concept of multi-molecular adhesion/decision cascades was confirmed in lymphocyte/monocyte-endothelial recognition systems (Bargatze *et al*, 1995). Recent studies suggest that differential patterns in the regulation of chemokines and their receptors likely determine the sequential steps of monocyte migration across the BBB (Weber *et al*, 1999).

In vitro systems for studies of leukocyte trafficking across the BBB

The need for reliable *in vitro* laboratory systems to study the mechanisms of cell trafficking into the brain is intuitively obvious. The difficulties in establishing such a system, however, abound. The BBB itself is complex (both physiologically and anatomically) (Pardridge, 1983). Modeling systems must take into account the cellular, matrix, immune and physiologic components of this complex system. The capillaries which form the BBB express a diverse array of functional characteristics that permit the regulation of cell, protein, and macromolecule passage between blood and brain (Pardridge, 1983). These are all difficult to recapitulate in laboratory cell systems. Nevertheless diverse BBB models have been developed and include: (1) monolayers of primary BMVEC; (2) co-cultures of astrocytes and endothelial cells derived from organs other than the brain; and (3) co-culture systems of astrocytes and BMVEC.

The first primary cultures of human BMVEC were established by Dorovini-Zis *et al* (1991) from microvessels of cortical fragments either removed at surgery, or from brains at autopsy. These monolayers expressed specific endothelial cell markers (factor VIII/Von Willebrand antigen and lectin-binding sites for *Ulex europaeus* agglutinin) and contained few pinocytotic vesicles. Transmission electron microscopy showed that endothelial cells were connected by tight junctions. The unique functional properties of the BMVEC are likely regulated by glial (most prominently astrocyte) secretory products (Janzer and Raff, 1987). Several studies have shown the importance of astrocyte-associated molecules in induction of BMVEC function [including expression of γ-glutamyltranspeptidase (γ-GTP), brain-type of glucose transporter (GLUT-1), and P-glycoprotein] (Hurwitz *et al*, 1993; Wolburg *et al*, 1994). It was suggested that endothelial cells isolated from different organs (lung, liver, umbilical cord and kidney) could acquire specific BMVEC markers when exposed to or co-cultured with astrocytes (Hurwitz *et al*, 1993). Hayashi *et al* (1997) constructed an *in vitro* BBB model where endothelial cells of human umbilical vein were cultured on one side of the porous membrane and rat fetal astrocytes were seeded on the other. Constructs where astrocyte endfeet were allowed to contact endothelial cells demonstrated tight junction formation. This is critical for barrier function limiting macromolecular transport (like [^{3}H]-inulin) and/or the expression of γ-GTP, GLUT-1, and P-glycoprotein. Additional works utilizing endothelial cells showed that a heat-labile factor from astrocytes increased barrier properties (detected as transendothelial electrical resistance) and expression of the tight junction protein (ZO-1) (Gardner *et al*, 1997).

Although heterologous systems (non-brain endothelium/astrocytes) can recapitulate, in part, the major properties of the BBB the complexities of neural cell interactions dictate the use of primary brain-derived endothelial and neuronal cells in any cell system. Hurwitz *et al* (1994) described an *in vitro* BBB model consisting of both human BMVEC and astrocytes. In these experiments, BMVEC retained specific cellular markers. In our works, an *in vitro* model of BBB, that allows analysis of the penetration of HIV-1 and blood-derived monocyte/macrophages into the brain was developed (Persidsky *et al*, 1997). This BBB system uses primary human BMVEC, matrix, and human fetal astrocytes. The endothelium and astrocytes are cultured on the

opposite sides of a collagen-coated membrane containing 3 μm pore-size tissue culture inserts. This allows the direct contact of end-feet of the astrocyte network into a monolayer of BMVEC. The BMVEC express factor VIII and form tight junctions with high electrical resistance and very low permeability to [^{3}H]-inulin. These systems, collectively, can be used to study the mechanisms of leukocyte migration across the BBB. They all support an active role of BMVEC in this process.

The concept of endothelial cell activation was first proposed in the late 1980s (Pober, 1988) and revolves around locally secreted pro-inflammatory polypeptides, cytokines [tumor necrosis factor alpha (TNFα), interleukin 1 (IL-1), IL-6, interferon gamma (IFNγ)] mediating the interactions of leukocytes and microvascular endothelial cells (Cavender *et al*, 1989; Stolpen *et al*, 1986). Vascular endothelial cells, at the site of immune reactions, undergo a number of morphological and functional alterations, including increased permeability, hypertrophy, accumulation of intracellular organelles and proliferation (Cavender *et al*, 1989). Activated endothelial cells found in inflamed tissues have a distinct morphology, express major histocompatibility complex antigens, and adhesion molecules (ICAM-1, VCAM-1, E-selectin and others) usually not found on resting endothelium (Carlos *et al*, 1991; Granger and Kubes, 1994).

Cytokines effect leukocyte-endothelial interaction and ultimately cell migration into the brain. Indeed, a number of cytokines secreted during diverse CNS pathologic processes (stroke, meningitis, multiple sclerosis, HIV-1 encephalitis) have been shown to regulate inflammatory cell migration into the brain. Since the presence of adhesion molecules is a critical step in leukocyte transendothelial migration, monolayers of BMVEC were used for studies of adhesion molecule expression after treatment with relevant cytokines or lipopolysaccharide (LPS) (Huynh and Dorovini-Zis, 1993; Wong and Dorovini-Zis, 1995, 1996). Stimulation of BMVEC with LPS and cytokines induced an upregulation of adhesion molecules. This stimulation was maximal following exposure to LPS, TNFα or IL-1. Minimal alterations were observed with IFNγ alone. Increase of adhesion molecule expression (ICAM-1, VCAM-1 and E-selectin) was concentration- and time-dependent. It was observed as early as 4 h and persisted for up to 48–72 h after cytokine exposure. The pattern of adhesion molecule expression was found to influence adhesion/migration of leukocytes. Indeed, significant augmentation of T lymphocyte adhesion with a 30-fold increase in transendothelial migration of cells was shown after TNFα exposure. In support of this notion, Hurwitz *et al* (1994), utilizing co-culture of BMVEC and astrocytes showed upregulation of adhesion molecules (VCAM-1, ICAM-1) upon stimulation with TNFα. The use of blocking antibodies (Ab) allowed evaluation of the function properties of adhesion molecules. For example, Ab to ICAM-1 and VCAM-1 significantly, but not completely, inhibited lymphocyte binding to activated BMVEC (Wong *et al*, 1999). While VCAM-1 was not utilized in leukocyte transendothelial migration, movement across activated BMVEC was blocked by Ab to ICAM-1 and significantly inhibited by Abs to PECAM-1 and E-selectin. Although penetration of primary HIV-infected monocytes through BBB was not studied, the authors detected enhanced binding of the HIV-1 infected promonocytic cell line (U937) to BMVEC when compared to uninfected cells. Yet, another study using monolayers of BMVEC showed that lymphocyte adhesion to and migration across the BBB was dependent on lymphocyte and endothelium activation and expression of adhesion molecule on endothelial cells and the corresponding ligands on T lymphocytes (Greenwood *et al*, 1995). These results suggest that adhesion/migration of T lymphocytes and monocytes across the cerebral endothelial barrier are defined, at least partially, by the immune secretory factors regulating endothelial cell activation.

The neuroimmune events that regulate leukocyte migration across an intact BBB were studied in our laboratories using a BBB model where BMVEC and astrocytes were cultured on the opposite sides of a porous membrane (as described above) (Persidsky *et al*, 1997). This model system was used specifically to study monocyte transendothelial migration as it might occur during HIV-1 encephalitis. In this model equal numbers of unstimulated or immune (LPS) activated human monocytes were added to the 'endothelial' compartment of the model. HIV-1 infected and control (uninfected) cells were used in replicate experiments to assess the influence of viral infection on transendothelial migration. Immune activation resulted in a profound increase (up to 20-fold) in the numbers of migrating cells. Interestingly, viral infection did not enhance monocyte migration (Figure 1A,B). The activated monocytes showed increased numbers of philopodia, lysosomes and vesicular Golgi complexes and expressed large levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-10). Application of immune activated monocytes (HIV-1 infected or uninfected) to the 'endothelial' compartment progressively changed BMVEC morphology (compatible with alterations in activated endothelium) and induced VCAM-1 expression. The cellular processes of the BMVEC were retracted and contained increased levels of intermediate filaments at sites of monocyte transmigration (Figure 1C,D). Intercellular junctions between neighboring BMVEC were preserved. Utilizing this BBB model we found that the state of immune activation (rather than HIV-1 infection)

dictated the ability of monocytes to traverse the BBB. Activation of BMVEC, during monocyte interactions, played an important role in the overall transmigration process.

Activation of BMVEC is commonly accompanied by prominent changes in cytoskeleton (intermediate filaments) and adhesion molecule expression. It was shown recently that the cytoplasmic domains of endothelial cell adhesion molecules are linked to the actin cytoskeleton (Yoshida *et al*, 1996). Thus, integrins expressed on lymphocyte or monocyte membranes could interact with the endothelial cell adhesion molecules inducing changes in the cytoskeleton of BMVEC. Indeed, in a recent study, the signaling pathways in brain endothelial cells and subsequent cytoskeleton alterations were examined after Ab ligation of endothelial ICAM-1 which mimics adhesion of lymphocytes to CNS endothelium (Adamson *et al*, 1999). ICAM-1 cross-linking resulted in a reorganization of the endothelial actin cytoskeleton to form stress fibres and activation of the small guanosine triphosphate (GTP)-binding protein Rho which was shown to regulate cytoskeletal organization in a number of different types of cells. The Rho GTPases are involved in actin cytoskeleton organization and signal transduction. Migration of stimulated T lymphocyte through BMVEC monolayers was inhibited following pretreatment of endothelium with cytohalasin D which depolymerized actin filaments. A specific inhibitor of Rho proteins applied to BMVEC significantly decreased the migration of T lymphocytes, endothelial Rho-GTP loading, and endothelial actin reorganization, without affecting lymphocyte adhesion to BMVEC. These data show that BMVEC are actively involved in facilitating T lymphocyte migration through the BBB, and this process involves ICAM-1-stimulated rearrangement of the endothelial actin cytoskeleton and functional Rho proteins.

Interestingly, we found prominent ultrastructural changes of astrocytes in the model where the active migration of monocytes and direct cellular contacts with astrocytes were detected. These were suggestive of functional changes and reminiscent of those in reactive astrocytosis (Norenberg, 1994). The cause(s) of such changes in astrocyte morphology are unclear and may be related to some secretory

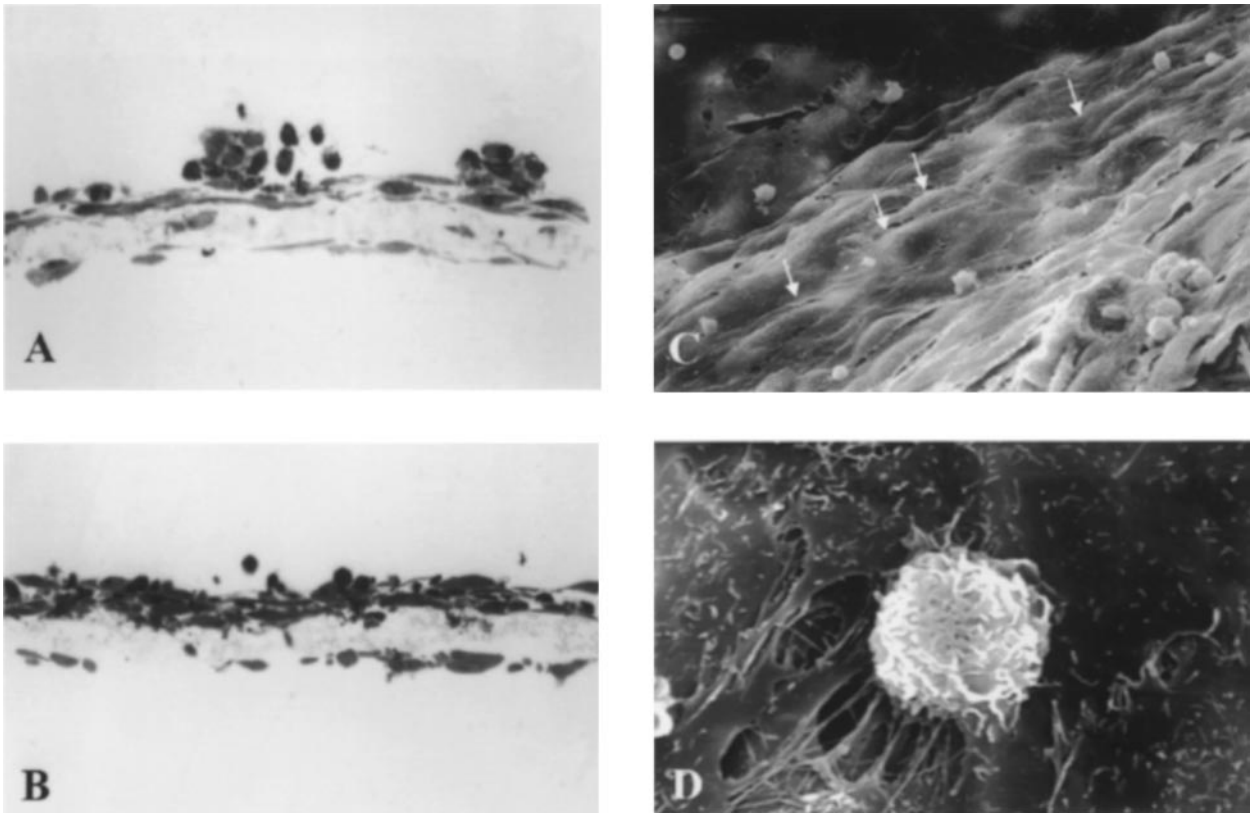

Figure 1 Migration of HIV-1 infected monocytes through the BBB model. (2 h after monocyte application). Monocytes (1.5×10^5 cell) were placed on the upper chambers of the models. While unstimulated cells added to upper chambers do not change BMVEC morphology (**A**), LPS-stimulated monocytes significantly cause retraction of endothelial cells and easily penetrate the model (**B**). Bulging of BMVEC (arrows) and gap formation are observed within monolayer with activated cells (**C**). Monocyte migration occurs into gaps between adjacent endothelial cells (**D**). **A,B** are semi-thin cross-sections of BBB model stained with toluidine blue. **C,D** are scanning electron microscopy of 'endothelial' surface of the model. Original magnification, **A,B** ×400; **C** ×800; **D** ×3500.

products of monocytes or endothelial cells since these changes were usually in areas where the most intense monocyte migration was found. Factors that can induce morphological changes in astrocytes include, but are not limited to, cytokines (IL-1, IL-6, TNFα, IFNγ) derived from microglia/astrocytes and adenosine triphosphate produced by endothelial cells (Kimelberg and Norenberg, 1994). Recent studies showed that reactive astrocytes are an important source of beta-chemokines in diverse neuro-inflammatory diseases (Glabinski and Ransohoff, 1999).

Chemokines are small molecular weight (7–10 kD) secreted proteins that influence recruitment and activation of leukocytes and other cells to sites of inflammation. Recent works in non-CNS models demonstrated that chemokine gradients promote transendothelial diapedesis of leukocytes into tissues stimulating directional activity (Luster, 1998). Each subfamily of chemokines has a specific variation of a conserved structural cysteine motif: alpha-subfamily (one amino acid separates the first two cysteine residues, CXC), beta- (cysteine residues are adjacent, CC), gamma- (only two cysteine residues are present, C) and delta- (three amino acids separate the first two cysteine residues, CX_3C) (Ransohoff, 1998). Members of a subfamily show considerable homology in amino acid sequence and overlapping cell-specific chemoattractive specificity. Alpha-chemokines [macrophage inflammatory protein two (MIP-2), interferon γ-inducible protein 10 kD (CRG-2/IP-10), IL-8, growth-related oncogene (GRO) α and β, C10] serve as chemotactic stimuli for polymorphonuclear leukocytes and lymphocytes. The β chemokines include: macrophage inflammatory proteins one alpha and one beta (MIP-1α and MIP-1β), macrophage chemotactic protein one (MCP-1), MCP-2, MCP-3, and regulated upon activation normal T cell expressed and secreted (RANTES). They promote migration of monocytes, lymphocytes, eosinophils and basophils. The patterns of chemokines secreted by different brain cells under the influence of a given stimulus or stimuli could explain the cellular composition of inflammatory infiltrates in brain tissue and pattern of tissue injury.

In vitro models could help to elucidate each chemokine contribution to leukocyte migration. To these ends, Weiss *et al* (1998) utilized a system with umbilical vein endothelial cells and fetal astrocytes on opposite sides of a porous membrane. They showed that MCP-1 was the primary chemoattractant for monocytes, and astrocytes (but not endothelial cells) stimulated by different pro-inflammatory cytokines were the major source of this chemokine. A blocking Ab to MCP-1 inhibited MCP-1- and cytokine-induced transmigration of monocytes by 85–90%. Using Ab to block adhesion molecules, Weiss *et al* (1998) showed that anti-ICAM-1 (completely) and anti-E-selectin (partially) blocked MCP-1 mediated monocyte migration across BBB, while anti-VCAM-1 had no effect. They also presented evidence that only activated lymphocytes migrated through blood–brain barrier constructs with or without pretreatment with cytokines. The order of potency was IL-1β or TNFα > IFNγ > > TGFβ. Contrary to monocytes, lymphocytes migrated more intensively through endothelial monolayers in absence of astrocytes, and only IFNγ induced significant lymphocyte migration above that of untreated cultures.

The interplay between divergent CNS cell types likely regulates chemokine secretory events in the brain. For example, BMVEC express chemokines and their receptors in addition to adhesion molecules and other pro-inflammatory products. Glial cells can respond to specific cytokines, to cell-cell interactions and/or activational factors and produce chemokines. In order to address these issues we studied the relationships between macrophage, microglial and astrocyte chemokine production and monocyte transendothelial migration into the brain. Monocyte entry into the brain during HIV-1 encephalitis served as our model system. An artificial BBB as described previously (Persidsky *et al*, 1997) where primary human BMVEC and astrocytes are placed on opposite sides of a matrix-coated porous membrane was used. In these more recent experiments microglia and/or monocyte-derived macrophages (MDM) were seeded in the bottom of the wells (Persidsky *et al*, in press). The transendothelial passage of monocytes (freshly purified from PBMC) was assessed after application of HIV-1-infected/uninfected MDM or microglia to the 'astrocyte' (brain) side of the BBB model. Microglia placed in the 'astrocyte' compartment induced increased monocyte migration across the BBB model, 2–3.5 times greater than that seen with MDM. HIV-1 infected microglia cells elicited a statistically significant increase monocyte migration *versus* uninfected microglia ($P < 0.013$). In order to explain such differences in migration, we assessed chemokine production in MDM and microglia. Activated microglia produced significantly more MCP-1 (as well as other β chemokines) than did similar numbers of MDM. HIV-1 infection did not affect chemokine levels in the immune-stimulated MDM or in microglia. Since this data was not concordant with the increased BBB monocyte migration shown with HIV-1-infected microglia, we evaluated the ability of MDM-astrocyte interactions to affect chemokine production. Indeed, addition of the macrophage supernatants to primary human fetal astrocytes induced astroglial chemokines. Interestingly, supernatants from virus-infected, activated microglia elicited the most significant levels of MCP-1. Our findings further support the idea of complex intercellular glial interactions in the neuropathogenesis of HIV-1 encephalitis. Such observations certainly apply to

other neuroimmune diseases where chemokines regulate leukocyte entry into the brain during progressive neurological disorders.

In vivo models for the studies of leukocyte recruitment into brain

The tissue culture systems described have certain limitations in adequately reflecting the complexities of cell to cell interactions *in vivo*, the intricacies of brain tissue function or regional brain differences in leukocyte migration. The pathogenesis of neuroimmune responses and associated BBB leukocyte migration can be studied comprehensively only *in vivo*. Several experimental approaches have been utilized in order to study leukocyte BBB migration including intracerebral injection of cytokines and chemokines into brains of rodents, transgenic mouse models with cytokine/chemokine expression within the CNS, and animal models recapitulating human neurological disorders. All of these have yielded important information suggesting that mononuclear cell recruitment into brain differs from that in peripheral tissues. For example, it was shown that following the intracranial injection of LPS or during acute neuronal degeneration, there is a paucity of polymorphonuclear leukocyte recruitment to the brain parenchyma and a delay in monocyte recruitment while there was a florid inflammatory response in meninges and choroid plexus (Andersson *et al*, 1991a,b; 1992a,b). Bell *et al* (1996b) investigated whether the injection of specific leukocyte chemoattractants into the murine CNS can override this intrinsic resistance. Recombinant alpha- (IL-8/NAP-1, MIP-2, IP-10) and beta-chemokines (MCP-1, RANTES) were injected into the murine hippocampus or subcutaneous tissue, and leukocyte accumulation was assessed. MCP-1 (1 μg) was found to be the most potent monocyte chemoattractant in the brain parenchyma and skin with IP-10 and RANTES producing minimal monocyte recruitment to both sites. IL-8 and MIP-2 injections resulted in significant polymorphonuclear leukocyte recruitment in both the central nervous system and skin. Breaching of the BBB was associated with polymorphonuclear leukocyte recruitment which was particularly prominent after injection of MIP-2. A recent study by Bolton *et al* (1998) demonstrated PMN accumulation and loss of BBB tight junction proteins after injection of IL-1β (100 units) into the striatum of juvenile rats (but not adult animals) raising a question of age-related differences in leukocyte migration into CNS. Concomitant changes in the structure of tight junctions were found by transmission electron microscopy. How specific these alterations might be to the given leukocyte cell type or type of neuroinflammatory stimulus awaits further investigation.

Transgenic mouse models with specific expression of the cytokines/chemokines offer another tool for the studies of leukocyte migration across the BBB *in vivo*. The role of TNFα in the pathogenesis of neurodegenerative disorders was studied in transgenic mice in which murine TNFα was expressed in astrocytes using a GFAP-TNFα fusion gene (Stalder *et al*, 1998). In two independent GFAP-TNFα transgenic lines (termed GT-8 and GT-2) adult (>4 months of age) animals developed a progressive ataxia (GT-8) or total paralysis affecting the lower body (GT-2). Mice had prominent meningoencephalitis (GT-8) or encephalomyelitis (GT-2) with predominantly perivascular accumulation of large numbers of B cells and $CD4^+$ and $CD8^+$ cells. The majority of these lymphocytes showed a memory cell phenotype and expressed an early activation marker (CD69). Contrary to perivascular infiltrates, parenchymal lesions were composed of mostly $CD45^+$, MHC class II^+, and Mac-1^+ cells of the macrophage/microglial lineage with few $CD4^+$ or $CD8^+$ T cells. Vascular expression of adhesion molecules (ICAM-1, VCAM-1, and MAdCAM) was upregulated and preceded the development of inflammation. A number of alpha- and beta-chemokines (MCP-1, MCP-3, C10, MIP-1β, RANTES and CRG-2) were expressed prior to inflammatory cell infiltration suggesting an important signaling role for these molecules in CNS leukocyte migration. Primary and secondary demyelination and neurodegeneration paralleled the development of inflammatory lesions. Interestingly, more extensive inflammatory lesions containing activated cells of the macrophage/microglial lineage, but mostly devoid of T lymphocytes occurred in GFAP-TNFα SCID mice. The presence of lymphocytes appears to counteract the recruitment or destructive potential of macrophages and microglia. The occurrence of a late-onset chronic inflammatory encephalopathy pointed to the existence of intrinsic CNS resistance to the recruitment and activation of leukocytes.

A pronounced mononuclear infiltrate is detected in transgenic mice expressing MCP-1 in oligodendrocytes under the control of the MBP promotor in the brain (Fuentes *et al*, 1995). Most recruited cells in the brain were monocytes and macrophages, as defined by light microscopy, and ultrastructural and immunohistochemical analyses. These cells were located in perivascular spaces with minimal infiltration into parenchyma. A predominant accumulation of MCP-1 in and around microvessels (as shown by immunohistochemistry) could explain such distribution. Interestingly, monocyte accumulation was significantly enhanced by intraparenchymal inoculation of LPS, suggesting that the recruitment properties of MCP-1 can be potentiated by additional factors. Notably, no neurological or behavioral changes were found in such mice

implying that other pro-inflammatory factors may be responsible for immune activation and parenchymal damage.

Tani *et al* (1996a) studied effects of the expression of α-chemokine KC in oligodendrocytes. Expression of KC resulted in significant accumulation of neutrophils colocalized with chemokine-expressing cells. Neurological impairment (postural rigidity and instability) developed at the fifth week after birth, and microglial activation and damage of BBB were the major neuropathologic changes at that time. *In toto*, transgenic models with chemokine expression in the brain suggested that chemokines are potent target-cell specific inducers of leukocyte migration across the BBB *in vivo*, and their activities are restricted to infiltration with minimal activation (Glabinski and Ransohoff, 1999).

A number of reports were focused on experimental autoimmune encepahlomyelitis (EAE), a model for multiple sclerosis where various chemokines were expressed in the brain resulting in lymphocyte and monocyte infiltration and demyelination (Glabinski *et al*, 1996, 1997; Tani *et al*, 1996b). Summarizing findings in chemokine gene expression and neuropathologic changes, Glabinski and Ransohoff (1999) admitted that chemokines (mainly expressed by astrocytes) amplify but do not initiate infiltration of leukocytes from the blood in acute EAE. Chronic relapses in this disease were associated with expression of a number of chemokines (MCP-1, IP-10, MIP-1α, GRO-α, RANTES). The functional significance of two chemoattractants, MIP-1α and MCP-1, in EAE pathogenesis was demonstrated when neutralization of these factors resulted in a conspicuous attenuation of disease (Karpus *et al*, 1995, 1997). While anti-MIP-1α Abs (not anti-MCP-1) blocked acute EAE achieved by adoptive transfer of T-cell blasts, anti-MCP-1 Abs (not anti-MIP-1α) diminished relapses of chronic EAE.

Abilities of T lymphocytes and macrophages to migrate across of basement membranes and to infiltrate neutrophil are ensured by the production of metalloproteinases (MMPs), proteolytic enzymes that are involved in the remodelling of extracellular matrix (Leppert *et al*, 1995). Thus, in EAE, MMP inhibitors might prevent the influx of inflammatory cells through the BBB (Clemments *et al*, 1997). Indeed, it has been shown that inhibition of MMP-9 activity in T lymphocytes is a major mechanism of action of IFNβ which is used for the treatment of multiple sclerosis (Stuve *et al*, 1997). Similarly, recruitment of T lymphocytes in the site of injury and damage of the BBB caused by the delayed-type hypersensitivity response in another animal model (simultaneous intracerebral and peripheral inoculations of heat-killed bacillus Calmette-Guerin) was reduced by a MMP inhibitor (Matyszak and Perry, 1996).

Animals with CNS viral infections feature recruitment of immune cells through the BBB into the brain. Recent studies showed T lymphocyte accumulation in mouse Sindbis virus infection (Irani and Griffin, 1996), lymphocytic choriomeningitis (Asensio *et al*, 1999) and mouse hepatitis virus encephalomyelitis (Lane *et al*, 1998). Though several β-chemokines were present in the brains of infected mice, only α-chemokine, IP-10/CRG-2, appear to correlate best with disease progression and lymphocyte recruitment. Overall, results obtained in EAE and different animal models for viral infections of the brain highlighted a pattern where multiple chemokine are expressed simultaneously suggesting interplay between glia (microglia/astrocytes), BMVEC and infiltrating leukocytes in neuroinflammation (Asensio *et al*, 1999). Further studies using mice with deleted chemokine receptors, application of neutralizing Abs or chemokine receptor antagonists will help to dissect the contribution of each chemokine in the process of leukocyte migration and overall progression of neuro-inflammation.

HIV-1 encephalitis (HIVE) is characterized by a significant infiltration of macrophages into the brain, formation of macrophage-derived multinucleated giant cells and microglial nodules, alteration of neuronal dendritic processes, a 30–50% decrease in large neurons in neocortex/deep grey matter, and astrogliosis (Gendelman *et al*, 1997; Koenig *et al*, 1986; Masliah *et al*, 1997; Wiley and Achim, 1994). Salient features of HIVE in humans were reproduced in SCID mice stereotactically inoculated with human monocyte-derived macrophages (MDM) infected with HIV-1 (Persidsky *et al*, 1996). Recently, possible pro-inflammatory and transendothelial migratory effects of human microglia were determined in the SCID mouse model for HIVE where human MDM and microglia (infected or uninfected) were stereotactically placed into SCID mouse brains. SCID mice received 1.5×10^5 HIV-1-infected or replicate uninfected microglia or MDM or monocyte culture media (control) into the basal ganglia (the region of brain tissue most affected in humans). At 7 days following inoculations equal numbers of HIV-infected microglia and monocytes were present in putamen (Figure 2A,C). Nearly 80% of MDM or microglia identified expressed HIV-1 p24 antigen (data not shown), and up to one-third of them were multinucleated (Figure 2A,C). Most of the human microglial cells preserved oval shape resembling activated ameboid microglial cells in HIVE. A pronounced accumulation of mouse macrophages (specifically labeled with *Griffonia simplicifolica* Lectin-Isolectin B_4) was found in and around the location of virus-infected human microglia. This was infrequently observed in mouse brains with HIV-1 infected MDM (Figure 2A,C). The extent and intensity of astrocyte reaction were more prevalent in mouse brains with

HIV-infected microglia as compared to MDM when assessed by image analysis at 7 days after inoculation ($P<0.02$) (Figure 2B,D). There was no accumulation of murine macrophages in the brains of control (media-inoculated) animals (Figure 2E), and only moderate increase of GFAP immunostaining was detected in astrocytes along the needle tract (Figure 2F). Interestingly, activation of microglia and diffuse microgliosis were previously shown to correlate with ventricular expansion and neuropathological changes in HIV-1-associated dementia (HAD) (Gelman, 1993; Tyor *et al*, 1992). These observations pointed to the existence of functional differences between resident brain macrophages as compared to blood-derived ones. Importantly, this animal model could serve as another model for the studies of monocyte infiltration into the CNS.

Previous works have demonstrated that simian immunodeficiency virus (SIV)-infected rhesus monkey model is an important system for studies of HAD in man. Indeed, it reproduces both neuropathologic changes and neurobehavioral

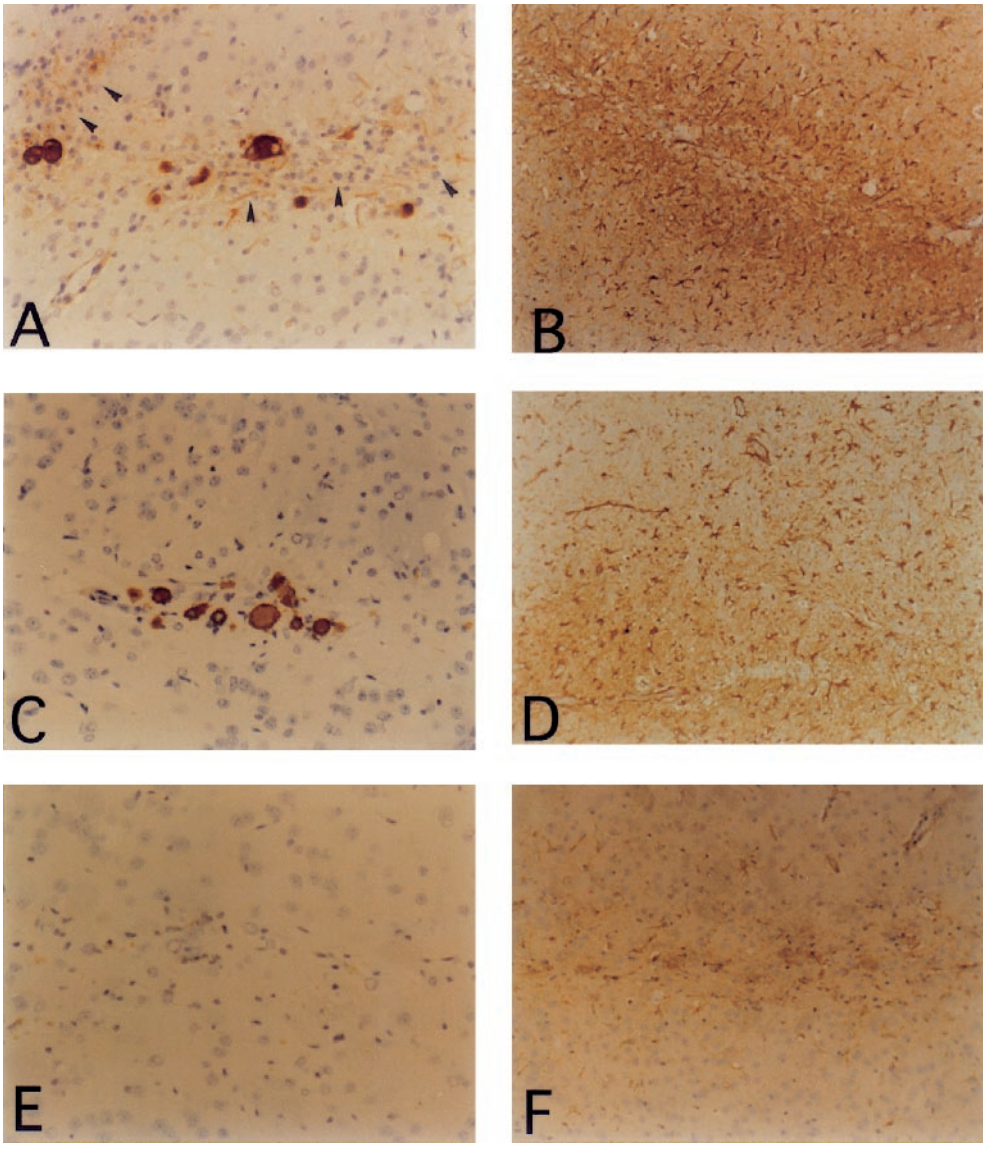

Figure 2 Microglia-mediated changes in SCID mice with HIV-1 encephalitis. Equal numbers of HIV-1 infected microglia (**A**) and monocytes (**C**) were steotactically inoculated into basal ganglia. Presence of human microglia elicited enhanced accumulation of mouse macrophages (**A**, arrowheads) that was absent in brain tissue inoculated with monocytes (**C**). More pronounced astrogliosis (GFAP immunostaining) was detected in areas contained microglia (**B**) as compared to monocytes (**D**). No inflammatory infiltrate was detected in the brains of sham-inoculated animals (**E**) with very mild GFAP reaction (**F**). **A,B,C,D,E** and **F** present serial coronal sections immunostained with anti-CS68 (**A,C,E**) and GFAP antibodies (**B,D,F**). Primary Abs are detected by Vectastain Elite Kit using DAB as a substrate. Tissue sections were counterstained with Mayer's hematoxylin. Original magnification, **A,C** and **E** $\times 400$, **B,D** and **F** $\times 200$.

abnormalities seen during HIV-1 infection (Rausch *et al*, 1999; Sasseville and Lackner, 1997). During peak viremia, rhesus macaques infected with pathogenic isolates of SIV have virus in the CSF and brain parenchyma by 2 weeks postinoculation. Circulating monocytes are a likely vehicle for cell-associated transport of virus across the BBB. The precise mechanisms responsible for SIV infection to the central nervous system remain illusive. Leukocyte recruitment, kinetics of perivascular macrophage/microglial turnover and associated transport of SIV in the CNS likely depend on endothelial and leukocyte interactions and expression of adhesion molecules and their ligands such as VCAM-1/VLA-4. VCAM-1 was shown previously to be upregulated on cerebrovascular endothelium in SIV encephalitis (Sasseville *et al*, 1994). The relationships between perivascular macrophage density, endothelial VCAM-1 expression and localization of viral nucleic acid was explored in the CNS of macaques acutely infected with SIV (Lane *et al*, 1996). Significant accumulation of perivascular macrophages expressing viral RNA by *in situ* hybridization coincided with viral neuroinvasion. Concomitantly, the CSF concentration of quinolinic acid, a marker of intrathecal immune and macrophage activation, was markedly elevated in CSF. These results support the idea that neuroinvasion occurs through an influx of infected monocytes which then reside in the CNS as perivascular macrophages. However, expression of VCAM-1 did not definitely correlate with macrophage infiltration. Same group investigated composition of chemokines in the encephalitic brain from SIV-infected animals (Sasseville *et al*, 1996). Enhanced expression of the C-C chemokines, MIP-1α, MIP-1β, RANTES, MCP-3, and the C-X-C chemokine, IP-10, was found by immunohistochemistry suggesting involvement of these chemokines in leukocyte recruitment to the brain in SIV-infected macaque monkeys. Recently, Westmoreland *et al* (1998) demonstrated the pattern of chemokine receptor expression in the SIV-infected rhesus macaque model. It was shown that the chemokine receptors, CCR3, CCR5, CXCR3, and CXCR4 are expressed in perivascular infiltrates in the SIV-infected brain tissues simian immunodeficiency virus (SIV). In addition, CCR3, CCR5, and CXCR4 were detected on subpopulations of large hippocampal and neocortical pyramidal neurons and on glial cells in both normal and encephalitic brain. These findings suggest that multiple chemokine receptors may contribute to monocyte and lymphocyte recruitment to the brain in SIV encephalitis. Similarly, chemokines likely play a critical role in the neuropathogenesis of HIVE. Schmidtmayerova *et al* (1996) showed chemokine mRNAs in cells with morphological features of macrophages/microglia in HIVE brain tissue. MCP-1 was also detected in brains and cerebrospinal fluid (CSF) of patients with HAD (Conant *et al*, 1998).

It is a well-known phenomenon that the inflammatory responses in the brain parenchyma almost always are restricted to mononuclear phagocytes and may be regulated by the trafficking T cells, but very rarely involve neutrophil responses. Preferential monocyte migration into the brain is supported by a number of studies including animal models for brain injury (brain trauma, neuronal damage induced by kainic acid and cerebral ischemia) (Andersson *et al*, 1991a; Ransohoff and Tani, 1998), Wallerian degeneration (reaction to the distal transection of axon) (Bruck, 1997; Griffin *et al*, 1996) or EAE (Glabinski *et al*, 1996, 1997; Tani *et al*., 1996b). Although causes of selective migration of monocytes to the brain remain elusive, recent works showing the significant role of macrophages in neuro-regeneration (Prewitt *et al*, 1997; Rapalino *et al*, 1998; Zeev-Brann *et al*, 1998) and their abilities to secrete neurotrophins may explain macrophage accumulation as a part of protective program triggered by CNS injury. Further exploration of this hypothesis and better understanding of macrophage neuroprotective phenotype could change our current view of the leukocyte migration to the brain as a harmful event.

Abbreviations

BBB, blood–brain barrier; BMVEC, brain microvascular endothelial cells; EAE, experimental autoimmune encephalomyelitis; HIVE, HIV-1 encephalitis; GFAP, glial fibrillary acid protein; γ-GTP, γ-glutamyltranspeptidase; GLUT-1, brain-type of glucose transporter; GRO, growth-related oncogene; ICAM, intercellular adhesion molecule; LFA, lymphocyte function-associated antigen; Mac-1, macrophage-1 antigen; MDM, monocyte-derived macrophage; MIP-1α, macrophage inflammatory proteins one alpha; MIP-1β, macrophage inflammatory proteins one beta; MCP-1, macrophage chemotactic protein one; MCP-2, macrophage chemotactic protein two; MCP-3, macrophage chemotactic protein three; IP-10, interferon γ-inducible protein; RANTES, regulated upon activation normal T cell expressed and secreted; MMP, metalloproteinases; SIV, simian immunodeficiency virus; VLA-4, very late activation antigen 4; VCAM-1, vascular cell adhesion molecule.

Acknowledgements

This work was supported in part by NIH grants R29 AI42404-02. I thank Dr HE Gendelman for careful review of the manuscript and for thoughtful discussions and Ms Robin Taylor for editorial support.

References

Adamson P, Etienne S, Couraud P, Calder V, Greenwood J (1999). Lymphocyte migration through brain endothelial cell monolayers involves signaling through endothelial ICAM-1 via a rho-dependent pathway. *Immunol* **162:** 2964–2973.

Andersson P-B, Perry V, Gordon S (1992b). The acute inflammatory to polysaccharide in CNS parenchyma differs from that in other body tissues. *Neurosci* **48:** 169–186.

Andersson P-B, Perry V, Gordon S (1991b). The CNS acute inflammatory response to excitotoxic neuronal cell death. *Immunol Lett* **30:** 177–181.

Andersson P-B, Perry V, Gordon S (1992a). Intracerebral injection of proinflammatory cytokines or leukocyte chemotaxins induces minimal myelomonocytic recruitment to the parenchyma of the central nervous system. *J Exp Med* **176:** 255–259.

Andersson P-B, Perry V, Gordon S (1991a). The kinetics and morphological characteristics of the macrophage-microglia response to kainic acid induced neuronal degeneration. *Neurosci* **42:** 201–214.

Asensio V, Kincaid C, Campbell I (1999). Chemokines and the inflammatory response to viral infection in the central nervous system with a focus on lymphocytic choriomeningitis virus. *J Neurovirol* **5:** 65–75.

Bargatze R, Julita M, Butcher E (1995). Distinct roles of L-selectin and integrins alpha 4 beta 7 and LFA-1 in lymphocyte homing to Peyer's patch-HEV in situ: the multistep model confirmed and refined. *Immunity* **3:** 99–108.

Bell M, Taub D, Perry V (1996b). Overriding the brain's intrinsic resistance to leukocyte recruitment with intraparenchymal injection of recombinant chemokines. *Neuroscience* **74:** 283–292.

Bolton S, Anthony D, Perry V (1998). Loss of tight junction proteins occludin and zonula occludens-1 from cerebral vascular endothelium during neutrophil induced blood-brain barrier breakdown in vivo. *Neurosci* **86:** 1245–1257.

Bruck W (1997). The role of macrophages in Wallerian degeneration. *Brain Pathol* **7:** 741–752.

Butcher E (1991). Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* **67:** 1033–1036.

Carlos T, Kovach N, Schwartz B, Rosa M, Newman B, Wayner E, Benjamin C, Osborn L, Lobb R, Harland J (1991). Human monocytes bind to two-cytokine-induced adhesive ligands on cultured human endothelial cells: endothelial-leukocyte adhesion molecule-1 and vascular cell adhesion molecule-1. *Blood* **77:** 2266–2271.

Carson M, Sutcliffe J (1999). Balancing function vs. self defensive: the CNS as an active regulator of immune responses. *J Neurosci Res* **55:** 1–8.

Cavender D, Edelbaum D, Ziff M (1989). Endothelial cell activation induced by tumor necrosis factor and lymphotoxin. *Am J Pathol* **134:** 551–560.

Clemments J, Cossins J, Wells G, Corkill D, Helfrich K, Wood L, Piggot R, Stabler G, Ward G, Gearing A, Miller K (1997). Matrix metalloproteinases expression during experimental autoimmune encephalomyelitis and effects of a combined matrix metalloproteinase and tumor necrosis factor-alpha inhibitor. *J Neuroimmunol* **74:** 85–94.

Conant K, Garzino-Demo A, Nath A, McArthur J, Halliday W, Power C, Gallo R, Major E (1998). Induction of monocyte chemoattractant protein-1 in HIV-1 Tat-stimulated astrocytes and elevation in AIDS dementia. *Proc Natl Acad Sci USA* **95:** 3117–3121.

Dorovini-Zis K, Prameya R, Bowman P (1991). Culture and characterization of microvascular endothelial cells derived from human brain. *Lab Invest* **64:** 425–436.

Fuentes M, Durham S, Swerdel M, Letwin A, Barton D, Megill J, Bravo R, Lira L (1995). Controlled recruitment of monocytes and macrophages to specific organs through transgenic expression of monocyte chemoattractant protein-1. *J Immunol* **155:** 5769–5776.

Gardner T, Lieth E, Khin S, Barber A, Bonsall D, Lesher T, Rice K, Brennan W (1997). Astrocytes increase barrier properties and ZO-1 expression in retinal vascular endothelial cells. *Invest Opthalmol Vis Sci* **38:** 2423–2427.

Gelman B (1993). Diffuse microgliosis associated with cerebral atrophy in the acquired immunodeficiency syndrome. *Ann Neurol* **34:** 65–70.

Gendelman H, Persidsky Y, Ghorpade A, Limoges J, Stins M, Fiala M, Morrisett R. (1997). The neuropathogenesis of HIV-1 dementia. *AIDS* **11** (Suppl A): S35–S45.

Glabinski A, Tani M, Strieter R, Tuohy V, Ransohoff R (1997). Synchronous synthesis of α- and β-chemokines by cells of diverse lineage in the central nervous system of mice with relapses of experimental autoimmune encephalomyelitis. *Am J Pathol* **150:** 617–630.

Glabinski A, Tani M, Tuohy V, Tuthill R, Ransohoff R (1996). Central nervous system chemokine gene expression follows leukocyte entry in acute murine experimental autoimmune encephalomyelitis. *Brain Behavior Immunity* **9:** 315–330.

Glabinski AR, Ransohoff R (1999). Chemokines and chemokine receptors in CNS pathology. *J NeuroVirol* **5:** 3–12.

Granger D, Kubes P (1994). The microcirculation and inflammation: modulation of leukocyte-endothelial cell adhesion. *J Leuk Biol* **55:** 662–675.

Greenwood J, Wang Y, Calder V (1995). Lymphocyte adhesion and transendothelial migration in the central nervous system: the role of LFA-1, ICAM-1, VLA-4 and VCAM-1. *Immunol* **86:** 408–415.

Griffin J, George E, Chaudhry V (1996). Wallerian degeneration in peripheral nerve disease. *Baillieres Clin Neurol* **5:** 65–75.

Hayashi Y, Nomura M, Yamagushi S-I, Harada S-I, Yamashita J, Yamamoto H. (1997). Induction of various blood–brain barrier properties in non-neural endothelial cells by close apposition to co-cultured astrocytes. *Glia* **19:** 13–26.

Hickey W (1997). Leukocyte migration into the central nervous system. In: *In Defence of the Brain: Current Concepts in the Immunopathogenesis and Clinical Aspects of CNS Infections.* Peterson P, Remington J (eds). Malden MA: Blackwell Science. pp 11–30.

Hurwitz A, Berman J, Lyman W (1994). The role of the blood–brain barrier in HIV-1 infection of the central nervous system. In: *Advances in Neuroimmunology.* San-Francisco CA. pp. 249–256.

Hurwitz A, Berman J, Rashbaum W, Lyman W (1993). Human fetal astrocytes induce the expression of blood–brain barrier specific proteins by autologous endothelial cells. *Brain Res* **625:** 238–243.

Huynh H, Dorovini-Zis K (1993). Effects of interferon-gamma on primary cultures of human brain microvessel endothelial cells. *Am J Pathol* **142:** 1265–1278.

Irani D, Griffin D (1996). Regulation of lymphocyte homing into the brain during viral encephalitis at various stages of infection. *J Immunol* **156:** 3850–3857.

Janzer R, Raff M (1987). Astrocytes induce blood–brain barrier properties in endothelial cells. *Nature* **325:** 253–257.

Karpus W, Kennedy K, Lucchinetti C, Bruck W, Rodriguez M, Lassman H (1997). MIP-1a and MCP-1 differentially regulate acute and relapsing autoimmune encephalomyelitis as well as Th1/Th2 lymphocyte differentiation. *J Leuk Biol* **62:** 681–687.

Karpus W, Lukas N, McRae B, Strieter R, Kunkel S, Miller S (1995). An important role for the chemokine macrophage inflammatory protein-1α in the pathogenesis of the T-cell-mediated autoimmune disease, experimental autoimmune encephalomyelitis. *J Immunol* **155:** 5003–5010.

Kimelberg H, Norenberg M (1994). Astroglial responses to CNS trauma. In: *The neurobiology of central nervous system trauma*. Faden S.S.a.A. (ed). New York: Oxford University Press.

Koenig S, Gendelman H, Orenstein J, Canto MD, Pezeshpour G, Yungbluth M, Janotta F, Aksamit A, Martin M, Fauci A (1986). Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science* **233:** 1089–1093.

Lane J, Sasseville V, Smith M, Vogel P, Pauley D, Heyes M, Lackner A (1996). Neuroinvasion by simian immunodeficiency virus coincides with increased numbers of perivascular macrophages/microglia and intrathecal immune activation. *J Neurovirol* **2:** 423–432.

Lane T, Asensio V, Yu N, Paoletti A, Campbell I, Buchmeister M (1998). Dynamic regulation of α- and β-chemokine expression in the central nervous system during mouse hepatitis virus-induced demyelinating disease. *J Immunol* **160:** 970–978.

Leppert D, Waubant E, Galardy R, Bunnet N, Hauser S (1995). T cell gelatinases mediate basement membrane transmigration in vitro. *J Immunol* **154:** 4379–4389.

Luster A (1998). Chemokines - chemotactic cytokines that mediate inflammation. *N Engl J Med* **338:** 436–445.

Masliah E, Hearton R, Markotte T, Ellis R, Wiley C, Mallory M, Achim C, McCutchan J, Nelson J, Atkinson J, Grant I (1997). Dendritic injury is a pathological substrate for human immunodeficiency virus-related cognitive disorders. HNRC Group. The HIV Neurobehavioral Research Center. *Ann Neurol* **42:** 963–972.

Matyszak M, Perry V. (1996). Delayed-type hypersensitivity lesions in the central nervous system are prevented by inhibitors of matrix metalloproteinases. *J Neuroimmunol* **69:** 141–149.

Meyer J, Rauh J, Galla H (1991). The susceptibility of cerebral endothelial cells to astrogial induction of blood–brain barrier enzymes depends on their proliferative state. *J Neurochem* **557:** 1971–1977.

Norenberg M (1994). Astrocyte response to CNS injury. *J Neuropathol Exp Neurol* **53:** 213–221.

Nottet H, Dhawan S (1997). HIV-1 entry into the brain: Mechanisms for the infiltration of HIV-1 infected macrophages across blood–brain barrier. In: *The Neurology of AIDS.* Gendelman H, Lipson S, Epstein L, Swidells S (eds). New York NY: Chapman & Hall. pp. 49–60.

Pardridge WM (1983). Brain metabolism: a perspective from the blood–brain barrier. *Physiol Rev* **63:** 1481–1535.

Persidsky Y, Limoges J, McComb R, Bock P, Baldwin T, Tyor W, Patil A, Nottet H, Epstein L, Gelbard H, Flanagan E, Reinhard J, Pirruccello S, Gendelman H (1996). Human immunodeficiency virus encephalitis in SCID mice. *Am J Pathol* **149:** 1027–1053.

Persidsky Y, Stins M, Way D, Witte M, Weinand M, Kim K, Bock P, Gendelman H, Fiala M (1997). A model for monocyte migration through the blood–brain barrier during HIV-1 encephalitis. *J Immunol* **158:** 3499–3510.

Pober J (1988). Cytokine-mediated activation of vascular endothelium. Physiology and pathology. *Am J Pathol* **133:** 426–433.

Prewitt C, Niesman I, Kane C, Houle J (1997). Activated macrophage/microglial cells can promote the regeneration of sensory axons into the injured spinal cord. *Exp Neurol* **148:** 433–443.

Ransohoff R (1998). Chemokines and CNS inflammation. *Neurotransmission* **14:** 3–12.

Ransohoff R, Tani M (1998). Do chemokines mediate leukocyte recruitment in post-traumatic CNS inflammation? *J Leuk Biol* **62:** 645–652.

Rapalino O, Lazarov-Spiegler O, Agranov E, Velan G, Yoles E, Fraidakis M, Solomon A, Gepstein R, Katz A, Belkin M, Hadani M, Schwartz M (1998). Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats. *Nature Medicine* **4:** 814–821.

Rausch D, Murray E, Eiden L (1999). The SIV-infected rhesus monkey model for HIV-1 associated dementia and implications for neurobiological diseases. *J Leuk Biol* **65:** 466–474.

Risau W, Woburg H (1990). Development of blood–brain barrier. *Trends Neurol Sci* **13:** 174–186.

Sasseville V, Lackner A (1997). Neuropathogenesis of simian immunodeficiency virus infection in macaque monkeys. *J Neurovirol* **3:** 1–9.

Sasseville V, Newman W, Brodie S, Hesterberg P, Pauley D, Ringler D (1994). Monocyte adhesion to endothelium in simian immunodeficiency virus-induced AIDS encephalitis is mediated by vascular cell adhesion molecule-1/alpha 4 beta integrin interactions. *Am J Pathol* **144:** 27–40.

Sasseville V, Smith M, Mackay C, Pauley D, Mansfield K, Ringler D, Lackner A (1996). Chemokine expression in simian immunodeficiency virus-induced AIDS encephalitis. *Am J Pathol* **149:** 1459–1467.

Schmidtmayerova H, Nottet H, Nuovo G, Raabe T, Flanagan C, Dubrovsky L, Gendelman H, Cerami A, Bukrinsky M, Sherry B (1996). Human immunodeficiency virus type 1 infection alters chemokine beta peptide expression in human monocytes: implications for recruitment of leukocytes into brain and lymph nodes. *Proc Natl Acad Sci USA* **93:** 700–704.

Stalder A, Carson M, Pagenstecher A, Asensio V, Kincaid C, Benedict M, Powell H, Masliah E, Campbell I (1998). Late-onset chronic inflammatory encephalopathy in immune-competent and severe combined immunodeficient (SCID) mice with astrocyte-targeted expression of tumor necrosis factor. *Am J Pathol* **153:** 767–783.

Stolpen A, Guinan E, Fiers W, Pober J (1986). Recombinant tumor necrosis factor and immune interferon act singly and in combination to reorganize human vascular monolayers. *Am J Pathol* **123:** 16–24.

Stuve O, Chabot Jung S, Williams G, Young V (1997). Chemokine-enhanced migration of human peripheral blood mononuclear cells is antagonized by interferon beta-through an effect on matrix metalloproteinase-9. *J Neuroimmunol* **80:** 38–46.

Tani M, Fuentes M, Peterson J, Trapp B, Durham S, Loy J, Bravo R, Ransohoff R, Lira S (1996a). Neutrophil infiltration, glial reaction, and neurological disease in transgenic mice expressing the chemokine N51/KC in oligodendrocytes. *J Clin Invest* **98:** 529–539.

Tani M, Glabinski A, Tuohy V, Stoler M, Estes M, Ransohoff R (1996b). In situ hybridization analysis of glial fibrillary acidic protein mRNA reveals evidence of biphasic astrocyte activation during acute experimental encephalomyelitis. *Am J Pathol* **148:** 889–896.

Tontsch U, Bauer H (1991). Glial cells and neurons induce blood–brain barrier enzymes in cerebral endothelial cells. *Brain Res* **539:** 247–253.

Tyor W, Glass J, Griffin J, Becker P, McArthur J, Bezman L, Griffin D (1992). Cytokine expression in the brain during the acquired immunodeficiency syndrome. *Ann Neurol* **31:** 349–360.

Weber K, Handelshausen PV, Clark-Lewis I, Weber P, Weber C (1999). Differential immobilization and heirarchical involvement of chemokines in monocyte arrest and transmigration on inflamed endothelium in shear flow. *Eur J Immunol* **29:** 700–712.

Weiss J, Downie S, Lyman W, Berman J (1998). Astrocyte-derived monocyte-chemoattractant protein-1 directs the transmigration of leukocytes across a model of the human blood–brain barrier. *J Immunology* **161:** 6896–6903.

Westmoreland S, Rottman J, Williams K, Lackner A, Sasseville V (1998). Chemokine receptor expression on resident and inflammatory cells in the brain of macaques with simian immunodeficiency virus encephalitis. *Am J Pathol* **152:** 659–665.

Wiley C, Achim C (1994). Human immunodeficiency virus encephalitis is the pathological correlate of dementia in acquired immunodeficiency disease syndrome. *Ann Neurol* **36:** 673–676.

Wolburg H, Neuhause J, Kniesel U, Krub B, Schmid E-M, Ocalan M, Farrell C, Risau W (1994). Modulation of tight junction structure in blood–brain barrier endothelial cells. *J Cell Sci* **107:** 1347–1357.

Wong D, Dorovini-Zis K (1995). Expression of vascular cell adhesion molecule-1 (VCAM-1) by human brain microvessel endothelial cells. *Microvasc Res* **49:** 325–339.

Wong D, Dorovini-Zis K (1996). Regulation by cytokines and lipopolysaccharide of E-selectin expression by human brain microvessel endothelial cells. *J Neuropathol Exp Neurol* **55:** 225–235.

Wong D, Prameya R, Dorovini-Zis K (1999). In vitro adhesion and migration of T lymphocytes across monolayers of human brain microvessel endothelial cells: regulation by ICAM-1, VCAM-1, E-selectin and PECAM-1. *J Neuropathol Exp Neurol* **58:** 138–152.

Yoshida M, Westlin W, Wang N, Ingber D, Rosenweig A, Resnik N, Gimborne M (1996). Leukocyte adhesion to vascular endothelium induces E-selectin linkage to the actin cytoskeleton. *J Cell Biol* **133:** 445–455.

Zeev-Brann AB, Lazarov-Spiegler O, Brenner T, Schwartz M (1998). Differential effects of central and peripheral nerves on macrophages and microglia. *Glia* **23:** 181–190.