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Review

Sentries at the gate: chemokines and the blood-brain barrier

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The blood-brain barrier and immune cell homing to the CNS

The blood-brain barrier (BBB) isolates the CNS from the rest of the organism. The anatomic foundation of this structure is a syncytium of cerebrovascular endothelial cells sealed together by tight junctions. The endothelial basal lamina, located at the abluminal side of endothelium, borders the perivascular (Virchow-Robin) space. The perivascular space is continuous with the subarachnoid compartment and is itself bordered by the glia limitans, formed by astrocytic and microglial end feet. Within the perivascular space, on either side of the endothelial basal lamina, dwell a unique population of phagocytic cells variously termed perivascular macrophages, perivascular microglia, perivascular cells or pericytes. These cells form a first line of defense once the BBB is breached. Astrocytes are also important for BBB development and function: their processes attach to the basement membrane shared with the endothelial cells and perivascular cells. The close contact between astrocytes and brain endothelial cells suggests that both cell types interact in maintaining the function of the BBB, a bidirectional interaction that is mediated at least in part by cytokine-mediated signaling (Selmaj, 1996; Johansson, 1990).

It is known that activated lymphocytes cross the intact BBB in the process of executing immunological surveillance of the CNS. T-cell migration into the normal CNS is antigen nonspecific and is contingent solely upon the activation state of the lymphocyte (Hickey *et al*, 1991; Wekerle *et al*, 1986). Leukocyte transmigration through a vascular endothelium involves a well-characterized multistep process of cell-cell interactions. Initial interactions between selectins on leukocytes and their receptors on endothelial cells slows down the circulating hematogenous cells, a state termed 'rolling'. Rolling is permissive for activation of adhesion molecules (leukointegrins), which promote tight adhesion of leukocytes to vascular endothelium. Finally inflammatory cells migrate through endothelium along concentrations gradients of chemokines (Springer, 1994).

Lymphocytes bind to CNS endothelial cells with lower affinity than to endothelium derived from other organs. After stimulation with proinflammatory cytokines lymphocyte adhesion to cerebrovascular endothelium increases and becomes similar to that observed in other organs (Male *et al*, 1990). Perhaps, this fact may explain why during CNS inflammatory conditions the BBB endothelium becomes permeable for hematogenous inflammatory cells.

Expression of the VLA-4 integrin by CD4 T cells is required for their migration into brain parenchyma in EAE. Moreover, antibodies to VLA-4 and its ligand VCAM-1 ameliorated pathogenicity of encephalitogenic cells *in vivo* (Baron *et al*, 1993; Yednock *et al*, 1992). A great deal of investigation of the BBB in inflammation has focused on EAE. After BBB penetration and local encounter with antigens, lymphocytes initiate inflammatory processes within the CNS parenchyma. It has been shown that there are two stages in homing of T lymphocytes to the CNS during EAE: initial selective and second nonselective, when clinical signs appear (Karin *et al*, 1993). Only a small number of inflammatory

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cells accumulating in the CNS at the onset of EAE are antigen-specific. Studies with radioactively labeled MBP-specific T cells showed that they represent only 1 to 4% of the inflammatory cell population in the CNS at the onset of EAE. Furthermore, labeled cells remained primarily within the perivascular compartment (Cross *et al*, 1990). The pathogenic role for antigen specific T cells may thus be exerted by promoting migration of antigen-nonspecific inflammatory cells from the blood to the CNS parenchyma.

Chemokines and their receptors – a brief overview

Chemokines are small (8–12 kD) chemoattractant cytokines which can stimulate directional migration of cells. Traditionally, the chemokine family can be divided into four separate subfamilies according to the position of the first two cysteines near the N-terminus. In addition to differences in structure, there are also functional differences between subfamilies. In CXC or alpha- chemokines, the first two cysteines are separated by one additional amino acid. In humans CXC chemokine genes are clustered on chromosome 4. They can be further divided into two groups; those with ELR (glutamate-leucine-arginine) motif preceding the first cysteine are primarily chemoattractant for neutrophils, while non-ELR alpha-chemokines attract activated lymphocytes and varied other cells. CC or beta-chemokines have the first two conserved cysteines adjacent to one another. Their genes are mainly clustered on chromosome 17 in humans and they attract principally mononuclear inflammatory cells. Recently two new chemokine subfamilies with one member each were described. The C or gamma-chemokine subfamily consists of lymphotactin, which is a potent T lymphocyte chemoattractant. Its gene maps to chromosome 1 in human genome. The single member of the CX3C or delta- chemokine subfamily, named fractalkine, has a unique structure because of the presence of three amino acids intervening between the first two cysteines. Fractalkine, unlike other chemokines, is a membrane bound protein with a chemokine domain at its N-terminus, which can be cleaved and may chemoattract mononuclear leukocytes (Luster, 1998; Rollins, 1997).

All chemokine receptors belong to the seven transmembrane spanning, G-protein coupled receptor family. So far, five CXC and nine CC chemokine receptors have been cloned (Rollins, 1997). Additionally, one receptor each for fractalkine and lymphotactin have been identified. Most of the chemokine receptors display overlapping specificities. Several CC and two out of five CXC chemokine receptors (CXCR2 and CXCR3) can bind more than one ligand. The only ligand specific chemokine receptors are CXCR1 (IL-8 receptor), CXCR4 (SDF-1 receptor) and CXXXCR1 (fractalkine receptor). With few exceptions, the signaling chemokine receptors are family specific. A major anomaly is a non-signaling receptor, termed DARC (Duffy Antigen Receptor for Chemokines). This receptor is localized on erythrocytes and postcapillary venules and binds both CXC and CC chemokines. As DARC does not signal upon binding ligands, its physiological function remains obscure. Several DNA viruses encode molecules homologous to chemokine receptors. Well known examples are *Herpesvirus saimiri* ECRF3 (encoding a protein similar to CXCR1 and CXCR2) and Cytomegalovirus. US28 encoding a polyvalent CCR1-like molecule. It has been suggested that mimicking chemokine receptors may be advantageous for viruses to inactivate self-defense mechanisms of the infected host (Ahuja *et al*, 1994; Ahuja and Murphy, 1993; Luster, 1998; Rollins, 1997).

Chemokines and nervous-system inflammation

Chemokine expression within CNS and PNS tissues is commonly observed in the context of inflammation. In the following subsections, we summarize several studies of chemokine expression and function in model and clinical neurological disorders. This information provides a foundation for considering how chemokine expression affects the function of the BBB.

Chemokines in autoimmune CNS inflammation

Any process damaging the BBB enables blood cellular and molecular components to gain access to the CNS and initiate inflammatory responses. This process occurs in mechanical, vascular and infectious injury to the CNS. Much more obscure is the mechanism leading to CNS inflammation without initial disruption of the BBB. This situation is observed at the early stages of autoimmune inflammation in multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE).

It has been proposed that the prime triggering factor for the initiation of autoimmune CNS inflammation consists of antigen-specific T cells. After BBB penetration they may stimulate astrocytres and other CNS cells to produce chemokines (Tani, 1994; Glabinski *et al*, 1995a). Additionally, encephalitogenic T cells produce abundant levels of MCP-1, MIP-1α and RANTES (Godiska *et al*, 1995; Kuchroo *et al*, 1993; Sun *et al*, 1997). This chemokine gradient favors migration of other mononuclear leukocytes from the circulation to the CNS compartment. Chemokine expression was detected in EAE only when inflammatory cells were already present in the CNS (Glabinski *et al*, 1995b). The influx of a large population of inflammatory

cells is necessary for disease manifestation as shown in animals with depletion of effector cells from the circulation (Brosnan *et al*, 1981).

Six years ago, the first studies described increased expression of some chemokines at the onset of EAE (Hulkower *et al*, 1993; Ransohoff *et al*, 1993). Chemokines MCP-1 and IP-10 were highly expressed in mouse and rat models of EAE. In the mouse model, astrocytes were the cellular source of chemokine expression as showed by *in situ* hybridization/immunohistochemistry colocalization studies (Ransohoff *et al*, 1993; Tani *et al*, 1996a,b). Later, additional chemokines were also detected in the CNS of animals shortly before clinical signs of EAE (Godiska *et al*, 1995) and expression of some chemokines correlated with the intensity of CNS inflammation (Glabinski *et al*, 1998). Our studies suggested that chemokines produced by astrocytes were responsible for amplification of inflammatory cell invasion of the CNS but not its initiation (Glabinski *et al*, 1995b). In chronic relapsing EAE (ChREAE) we observed increased expression of several chemokines during spontaneous relapse of the disease as compared with disease remission. Some chemokines (MCP-1, IP-10 and GRO-α) were expressed by astrocytes, other (RANTES, MIP-1α) by inflammatory cells (Glabinski *et al*, 1997). Fractalkine and its receptor were also upregulated in EAE (Jiang *et al*, 1998; Pan *et al*, 1997). Expression of some chemokines by CNS parenchymal cells in the EAE model is probably stimulated by proinflammatory cytokines as shown by studies in mice with interferon gamma knock-out and EAE. In that model IP-10 expression was selectively diminished in contrast with two other chemokines, MCP-1 and GRO-α (Glabinski *et al*, 1999). The importance of chemokines in EAE pathogenesis was confirmed by experiments showing disease amelioration after treatment with anti-chemokine antibodies (Karpus and Ransohoff, 1998; Kennedy *et al*, 1998; Karpus and Kennedy, 1997; Karpus *et al*, 1995). In these studies, anti-MIP-1α antibody inhibited development of acute EAE, whereas anti-MCP-1 antibody reduced the severity of relapsing EAE. An important complementary study showed increased expression of chemokine receptors CCR2, CCR5, CXCR4, CXXXCR1 in the spinal cords of animals with EAE (Jiang *et al*, 1998).

Recent reports addressed expression of chemokines within the CNS during MS. MIP-1α concentrations in the CSF were statistically different from control during MS relapse and other inflammatory neurological diseases but in all cases was present at sub-picomolar levels (Miyagishi *et al*, 1995). MCP-1 was found in inflammatory cells and astrocytes in active plaques and astrocytes in surrounding white matter. Moreover, MIP-1α and MIP-1β were present in inflammatory cells in active MS plaques (Simpson *et al*, 1998). MCP-1, MCP-2 and MCP-3 were also detected in reactive astrocytes and inflammatory cells in active acute and chronic MS lesions (McManus *et al*, 1998a). RANTES was localized by ISH to perivascular inflammatory cells (Hvas *et al*, 1997).

We found increased expression of chemokines IP-10, Mig and RANTES in cerebrospinal fluid from patients with MS relapse (Sørensen *et al*, 1999). Additionally, we localized cells bearing receptors for those chemokines within MS plaques. CXCR3 (IP-10/Mig receptor) was present on lymphocytes within perivascular inflammatory cuffs; CCR5 (a RANTES receptor) was present on lymphocytes, macrophages and microglial cells in active MS lesions (Sørensen *et al*, 1999). Compatible results were reported by Balashov *et al* (1999), who additionally demonstrated increased production of IFN-γ by circulating CCR5+ T cells.

The implications of studying chemokines and their receptors in CNS immune-mediated inflammation are only provisional at best. The tight relationship between disease activity and chemokine expression in EAE is impressive, as is the correspondence between populations of infiltrating cells and production of appropriate chemokines. Abrogation or modulation of disease by anti-chemokine antibodies also supports the role of these cytokines in EAE pathogenesis. The recent results obtained from studying MS imply the possibility of using chemokine receptor blockade to treat this serious and often disabling disorder. However, the specific roles of chemokines and their receptors in this complex pathology (chemoattraction; activation; mitogenesis) remain to be defined.

Chemokines in physical and chemical CNS injury

Chemokines are expressed in the CNS in diverse models of physical insult. Astrocytes were the main producers of MCP-1 shortly after penetrating mechanical injury to the brain (Berman *et al*, 1996; Glabinski *et al*, 1996). This expression was detected at the level of message as well as protein (Berman *et al*, 1996; Glabinski *et al*, 1998) and was localized on astrocytes in the vicinity of the lesion within hours after trauma (Glabinski *et al*, 1996). Other chemokines were not expressed at detectable levels in that injury model. Astrocytic expression of MCP-1 preceded influx of inflammatory cells to sites of injury and was present only in models with subsequent inflammatory reaction; thus, we observed only minimal expression of MCP-1 in a neonatal stab injury model characterized by lack of inflammatory cell migration to the injury site (Glabinski *et al*, 1996). In other studies, RANTES and MIP-1β were detected 1 day after stab injury to the brain, using immunohistochemistry which localized MIP-1β in reactive astrocytes and macrophages at the site of injury, while RANTES was diffusely expressed in necrotic tissue (Ghirnikar *et al*, 1996). When cortical lesion was augmented with lipopolysaccharide (LPS), many chemokines (MIP-

1α, MIP-1β, RANTES, IP-10, KC) were expressed in addition to MCP-1 (Hausmann *et al*, 1998). Neutralization of MCP-1 activity after stab wound injury to the brain with antisense oligodeoxynucleotides reduced by 30% the number of accumulated macrophages, demonstrating the potential of therapeutic modulation of posttraumatic inflammation in the brain using anti-chemokine strategies (Ghirnikar *et al*, 1998). Myelin edema chemically induced by triethyltin (TET) was shown to be associated with very rapid overexpression of proinflammatory cytokines including MIP-1α (Mehta *et al*, 1998).

In experimental spinal cord injury MIP-1α and β were upregulated biphasically. In the early phase, 24 h after injury, chemokine expression was diffuse within the spinal cord gray matter. Later (4 days after injury), chemokine expression was restricted to inflammatory cells in proximity to the lesion (Bartholdi and Schwab, 1997). Cryogenic injury to the brain also increased expression of MCP-1 in the injured hemisphere, with a peak at 6 h post-injury and return to baseline by 48 h (Grzybicki *et al*, 1998). In the unlesioned hemisphere MCP-1 was also expressed, albeit to a lesser extent. We did not observe expression of IP-10 in this injury model (Grzybicki *et al*, 1998).

In a clinical study, CSF IL-8 concentrations were significantly higher in patients with severe traumatic brain injury than in corresponding sera, suggesting intrathecal production of this chemokine. CSF IL-8 levels correlated with BBB dysfunction measured by CSF/serum albumin ratio and nerve growth factor (NGF) production, suggesting an important role for IL-8 in the pathogenesis of brain injury (Kossmann *et al*, 1997).

It is notable that chemokines are rapidly, selectively and abundantly made in traumatized neural tissue, by resident cells such as astrocytes and Schwann cells. The study of chemokines in posttraumatic inflammation holds significant promise for devising methods to ameliorate the burden of trauma-associated disability. It will probably be possible to devise models of trauma that lack production of chemokines (in chemokine-deficient, gene-targeted mice) or response to chemokines (in receptor knockouts). These models will help to determine in which situations chemokine reduction or chemokine receptor blockade will be beneficial. In some select circumstances, the production of chemokines may be beneficial, and could be therapeutically augmented or substituted by chemokine mimicks.

Chemokines in vascular CNS injury

Increased expression of chemokines in the CNS was observed in varied experimental models of cerebral ischemia. Locally expressed in ischemic brain tissue, chemokines may be responsible for attraction of inflammatory cells and may contribute to tissue injury. In an early study, increased mRNA expression for MCP-1 and MIP-1α was detected as early as 6 h after onset of cerebral ischemia (Kim *et al*, 1995). MCP-1-positive cells had the morphology of endothelial cells and macrophages, while MIP-1α-positive cells resembled astrocytes (Kim *et al*, 1995). Others also observed increased MCP-1 expression in the ischemic cortex beginning 6 h after middle cerebral artery occlusion (MCAO) (Wang *et al*, 1995). From 6 h to 2 days after MCAO astrocytes surrounding the ischemic tissue were the main cellular source of MCP-1; after 4 days MCP-1 was detected in macrophages and microglia in the ischemic area (Gourmala *et al*, 1997). MIP-1α expression was increased 4–6 h after experimental brain ischemia and localized in microglial cells within the ischemic region (Takami *et al*, 1997). Expression of the rat GAO-α homolog CINC (cytokine-induced neutrophil chemoattractant) was increased in the ischemic cerebral cortex 6–12 h after MCAO. No significant expression of CINC was detected in contralateral cortex (Liu *et al*, 1993). Interestingly, serum level of CINC was also transiently elevated 3–6 h after brain reperfusion injury (Yamasaki *et al*, 1995). In neonatal model of hypoxic-ischemic injury to the brain MCP-1 was first detected in periventricular regions of the lesioned hemisphere 1 h after ischemia. Peak of mRNA expression was observed at 8–24 h, and completely resolved by 48 h (Ivacko *et al*, 1997).

Reperfusion after transient focal brain ischemia induces accumulation of neutrophils and severe brain edema. Depletion of neutrophils diminished the size of damaged area showing that neutrophils are primary mediators of reperfusion injury (Romson *et al*, 1983). Expression of neutrophil chemotactic cytokines IL-8 and CINC was shown to be increased after reperfusion (Matsumoto *et al*, 1997; Yamasaki *et al*, 1995). Administration of anti-IL-8 antibody significantly reduced brain edema and the volume of infarcted tissue (Matsumoto *et al*, 1997). Increased MCP-1 expression was also reported in rat forebrain reperfusion model, and was detected by RT/PCR as early as 1 h after reperfusion (Yoshimoto *et al*, 1997). Clinical studies showed that MCP-1 and IL-8 levels were evaluated in the CSF of patients operated for intracranial aneurysms. In patients with unruptured aneurysms, chemokine level was very low but increased significantly after subarachnoid haemorrhage (Gaetani *et al*, 1998).

In common with other determinants of cellular infiltration and activation (such as adhesion molecules), chemokines are considered potential therapeutic targets in cerebral ischemia. No 'proof-of-principle' experiments have been reported, however.

Chemokines in CNS infections

Clearly chemokine production by CNS and PNS cells has the evolutionary advantage of providing

rapid and selective accumulation of leukocytes to a site of infection. Thus, the role of chemokines in host defense against pathogens has been characterized in multiple models of neural and extraneural infection. Not surprisingly, much of this effort has been devoted to understanding the beneficial and pathogenic roles of chemokines in the setting of chronic infection with HIV and related viruses.

Viral infection of the CNS parenchyma strongly stimulates local chemokine expression. In a nonhuman primate model of AIDS encephalitis induced by simian immunodeficiency virus (SIV) increased expression of chemokines MIP-1α, β, RANTES, MC-3 and IP-10 was observed in infected brains. Chemokines were expressed mainly in endothelial cells and perivascular monocytes/microglia (Sasseville *et al*, 1996). Increased expression of RANTES mRNA was detected in trigeminal ganglia taken from mice from five through 135 days after infection with herpes simplex virus type 1 (Halford *et al*, 1996). Histological evaluation indicated that only a very few mononuclear cells remained in the ganglion several days after infection despite the presence of HSV-1 latency-associated transcripts (Halford *et al*, 1996).

In brains from mice with lymphocytic choriomeningitis transcripts for chemokines, including IP-10, RANTES, MCP-1, MIP-1β and MCP-3, were detected by day 3 postinfection. Three days later mRNA for lymphotactin, C10, MIP-2 and MIP-1α was present (Asensio *et al*, 1999; Asensio and Campbell, 1997). Mice infected with mouse hepatitis virus (MHV) develop acute encephalomyelitis and chronic demyelinating disease. At the time of disease manifestation, mRNA for chemokines CRG-2/IP-10, RANTES, MCP-1, -3, MIP-1β and MIP-2 was detected in brains and spinal cords. Astrocytes expressed IP-10 in this model (Lane *et al*, 1998). Increased IL-8 activity was observed in CSF from dogs with acute and chronic stages of canine distemper virus (CDV) infection characterized by multifocal demyelination in the central nervous system (Tipold *et al*, 1999). Mouse adenovirus -type 1 (MAV-1) causes fatal haemorrhagic encephalopathy associated with increased expression of IP-10, MCP-1, MIP-1α, MIP-1β and RANTES in the CNS of C57BL/6 mice. IP-10 expression was detected in that disease by *in situ* hybridization on endothelium and CNS glia (Charles *et al*, 1999).

HIV-associated dementia is characterized by monocytic infiltration of the CNS. This feature suggested that chemokines can be involved in the pathomechanism of that disease. It has been shown that HIV transactivator protein Tat stimulates expression of MCP-1 by cultured astrocytes. Other chemokines were not detected in those cultures. Moreover, MCP-1 expression was detected in the brains and CSF of patients with HIV-1 associated dementia (Conant *et al*, 1998). In brains from patients who died with HIV encephalitis chemokines MCP-1, MIP-1α and RANTES were detected on macrophages (Sanders *et al*, 1998).

Intracisternal administration of anti-MIP-1 (including β-chemokines MIP-1α and MIP-1β) and anti-MIP-2 (α-chemokine similar to human GRO-α) antibodies during induction of experimental pneumococcal meningitis delayed the onset of CSF inflammation (Saukkonen *et al*, 1990). In brains from mice with experimental *Listeria* meningoencephalitis, transcripts for MIP-1α, β and MIP-2 were detected by Northern analysis 24 h after infection (Seebach *et al*, 1995). MIP-1α-positive cells were first seen in the lateral and third ventricles 12 h after disease induction. The vast majority of those cells were neutrophils. MIP-2 positive cells were seen later (24–72 h). At 72 h after disease induction, neutrophils and macrophages expressed both types of chemokines. Increased levels of MIP-1α and MIP-2 were detected by ELISA in CSF from infected animals with the peak at 48–72 h post infection (Seebach *et al*, 1995). Interpretation of these studies is complicated by the subsequent findings that MIP-1 contained two distinct chemokines (MIP-1α and MIP-1β) and the atypical properties of murine MIP-1α, which can serve as a neutrophil chemoattractant.

In the CSF of patients with pyogenic meningitis elevated levels of IL-8, GRO-α, MCP-1, MIP-1α and β were found (Spanaus *et al*, 1997). These patients suffered from pneumococcal, meningococcal and Haemophilus influenzae bacterial meningitis. Chemotactic activity of CSF for neutrophils was reduced by anti-IL8 and anti-GRO-α antibodies. Similarly, mononuclear cell migration was diminished by a combination of anti-MCP-1, anti-MIP-1α and anti-MIP-1β antibodies (Spanaus *et al*, 1997). In another study IL-8 level was increased in CSF from all patients with pyogenic meningitis but only in a few with aseptic meningitis. There was significant correlation between IL-8 level and CSF neutrophil counts in patients with nonpyogenic meningitis (Lopez-Cortes *et al*, 1995). Others observed relationship between CSF IL-8 and GRO-α levels and granulocyte counts in that compartment in patients with bacterial meningitis and between MCP-1 CSF concentration and mononuclear cell counts in CSF from patients with non-bacterial meningitis (Sprenger *et al*, 1996).

The therapeutic implications of chemokine expression in CNS infections are as protean as the agents responsible. Experiments using mice deficient in MIP-1α indicated severe defects for response to viral infection in the lung, but decreased cardiac pathology after *Coxsackie B* virus infection. Further, MIP-1α knock-out mice infected with fungus *Cryptococcus neoformans* had decreased leukocyte recruitment and crypto-

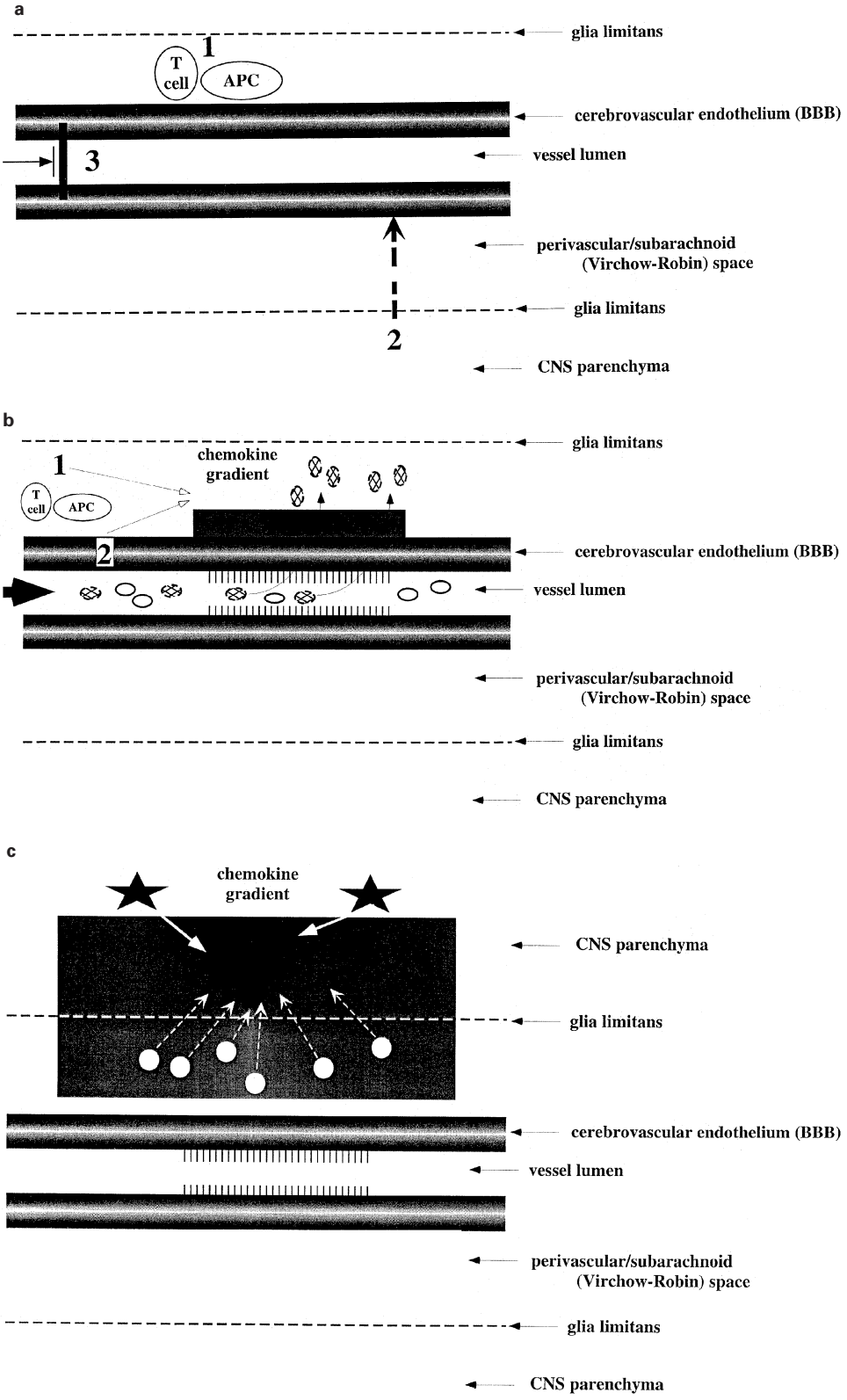

Figure 1 **(a)** Multiple pathways by which chemokine expression is initiated near the BBB. The cartoon shows the BBB and associated CNS structures, including the perivascular space, the glia limitans and the parenchyma. In all figures, the direction of blood flow is left-to-right. Representative pathological events are depicted. Chemokine expression can result from varied insults: (1) during immune-mediated inflammation, antigen presentation and recognition by T cells and APCs causes chemokine production in the perivascular space, behind the BBB; (2) mechanical trauma (dashed arrow) directly impacts cellular elements of the vasculature, leading to chemokine production; (3) arterial occlusion (solid rectangle with blocked arrow) with resulting ischemic injury damages the vascular

coccal clearance from the brain (Huffnagle and McNeil, 1999), showing the importance of chemokines in the host defense against CNS fungal infection. Cryptococcal infection stimulates IL-8 production by cultured brain glial cells and high levels of IL-8 were found in the CSF of patients with cryptococcal meningitis (Lipovsky *et al*, 1998). It must be recalled that expression of IL-8 by human patients with cryptococcal infection may be functionally analogous to expression of murine MIP-1α, since this β-chemokine can serve as a neutrophil chemoattractant. As a general comment, attempts to modulate the outcome of *acute* viral, bacterial or fungal infection by chemokine blockade will be attended by concern about impaired host defence. More promising is evaluation of the potential roles of chemokines and chemokine receptors in *chronic* viral infection, as seen in patients with HIV encephalopathy.

Chemokines and the BBB

All CNS cell types crucial for proper BBB functioning are able to express chemokines *in vitro*. Brain endothelial cells can produce MCP-1 and IL-8, with expression increased after stimulation with TNF-α or infection by bacterial parasites (Bourdoulous *et al*, 1995; Zach *et al*, 1997). Cultured normal and neoplastic astrocytes express chemokines including MCP-1 (Hayashi *et al*, 1995; Morita *et al*, 1993; Yoshimura *et al*, 1989), IL-8 (Morita *et al*, 1993; Nitta *et al*, 1992), IP-10 (Glabinski *et al*, 1999), MIP-1α and β (Miyamoto and Kim, 1999; Peterson *et al*, 1997) as well as RANTES (Barnes *et al*, 1996). TNF-α and IL-1β stimulate expression of MCP-1 and RANTES by primary human astrocytes. IFN-γ selectively induces IP-10 expression in such cultures (Oh *et al*, 1999). TGF beta and IL-10 down-regulate expression of MCP-1, MIP-1α, MIP-1β and RANTES by stimulated astrocytes (Guo *et al*, 1998). Microglia can produce IL-8, MCP-1, MIP-1α and β when cultured *in vitro* with inflammatory cytokines (Erlich *et al*, 1998; Hayashi *et al*, 1995; McManus *et al*, 1998b).

Recent studies showed that anti-MCP-1 antibody inhibited transmigration of monocytes across untreated and IL-1-stimulated cultured endothelial cells. In this system, MCP-1 stimulates transmigration of monocytes only when there is a concentration gradient across the endothelial monolayer with higher concentration in the direction of migration (Randolph and Furie, 1995). Recent studies with a model of the BBB showed that MCP-1 could induce the migration of monocytes and lymphocytes through a coculture of human endothelial cells and astrocytes. In this elegant system, stimulation with proinflammatory cytokines like TNF-α, IFN-γ and IL-1β increased monocyte transmigration, which was significantly reduced by anti-MCP-1 antibodies (Weiss *et al*, 1998).

Transgenic expression of chemokines behind an intact BBB, governed by an oligodendrocyte-specific promoter, showed that chemokines could direct the accumulation of specific populations of leukocytes within the brain. Specifically, MCP-1 expression under control of the MBP promoter, induced migration of monocytes/macrophages (Fuentes *et al*, 1995). Immunostaining detected the presence of this chemokine at the abluminal surface of cerebrospinal microvessels and infiltrating cells were localized mainly in the perivascular space (Fuentes *et al*, 1995). MBP-directed transgenic expression of GRO-α (a potent neutrophil chemoattractant) produced massive accumulation of neutrophils in perivascular, meningeal and parenchymal CNS sites. GRO-α immunoreactivity was detected in oligodendrocytes and colocalized with infiltrating neutrophils (Tani *et al*, 1996b).

Intraparenchymal chemokine expression, directed by adenoviral vectors, mediated selective recruitment of either monocytes or neutrophils depending on which chemokine was expressed (Bell *et al*, 1996). In these experiments, IL-8 and MIP-2 induced neutrophil accumulation, whereas MCP-1 was a potent monocyte chemoattractant. Migration of inflammatory cells correlated in these studies with BBB breakdown (Bell *et al*, 1996). Inflammatory cytokines IL-1 and TNF-α injected intracerebrally did not induce recruitment of monocytes into the brain parenchyma. Interest-

bed, causing chemokine production and BBB disruption. APC: antigen-presenting cell. (**b**) Simultaneous establishment of chemokine gradients and activated endothelium promote leukocyte migration across the BBB. Most insults that lead to chemokine production also upregulate adhesion molecules (vertical bars) on the luminal aspect of the endothelium. Signals that direct chemokine expression and adhesion molecule upregulation can derive either from (1) immune-mediated inflammation or 2: direct damage to the vessel wall and vascular endothelium. In either case, leukocytes (hatched circles) that express appropriate ligands for endothelial adhesion molecules (vertical bars) and for chemokine gradients in the microenvironment (shaded rectangle) will encounter conditions that favor extravasation. Leukocytes (open circles) lacking such receptors remain in the following bloodstream. (**c**) Leukocytes in the CNS perivascular space elicit chemokine expression by nearby glia and also respond to these chemokines. Perivascular leukocytes (white circles) are typically highly activated and produce inflammatory cytokines. These secreted products stimulate nearby astrocytes (star-shaped cells) to express chemokines such as IP-10 and MCP-1, establishing a chemokine gradient (shaded rectangle) across the glia limitans. These chemokines deliver both chemoattractant and activating signals to leukocytes that express appropriate receptors. These conditions favor invasion of CNS parenchyma by leukocytes (dashed arrows) as well as persistent inflammatory microenvironment in the perivascular compartment.

ingly, these cardinal inflammatory cytokines produced neutrophil margination around vessels but not deeper migration into the brain parenchyma, showing that margination and parenchymal invasion may be regulated by distinguishable influences (Andersson *et al*, 1992).

Chemokines, BBB and initiation of CNS inflammation

Any cell that migrates from the blood to the CNS parenchyma has to do so in two steps. First, it transmigrates in a strictly controlled way from the vascular lumen across the BBB into the perivascular subarachnoid space (Figure 1). Second, cells within the perivascular/subarachnoid space can either remain resident there, re-enter the bloodstream (or undergo apoptosis), or can enter the CNS parenchyma. Cerebrospinal fluid (CSF) components reflect the content of the CNS extracellular fluid, consistent with the concept that the CSF–CNS barrier (glia limitans) is less restrictive than the BBB. However, the molecular mechanisms that govern infiltration of cells from the perivascular/subarachnoid space into the parenchyma remain almost completely undefined.

Chemokines may exert important functions during multiple steps of inflammatory cell extravasation (Figure 1). During the first step, chemokines immobilized on the luminal surface of the endothelium may signal to circulating leukocytes. Moreover, chemokines 'presented' by endothelial cells may stimulate expression and activation of adhesion molecules on target leukocytes and promote their attachment to the vascular endothelium (Figure 1).

When produced abluminally by CNS parenchymal cells, chemokines must be internalized and transcytosed through the endothelial cell cytoplasm to the luminal surface (Middleton *et al*, 1997). This concept addresses the problem that a soluble chemokine gradient cannot be maintained across an endothelium in contact with circulating blood (Tanaka *et al*, 1993). IL-8 immunoreactivity was shown by electron microscopy on luminal projections of endothelial cells after microinjection into rabbit skin (Middleton *et al*, 1997). It was suggested that heparan sulfate present on the endothelial cell-surface immobilized chemokines (Rot, 1992). This hypothesis was supported by the observation that heparitinase, an enzyme that specifically hydrolyses heparan sulfate, reduced endothelial cell-associated IL-8 immunoreactivity (Middleton *et al*, 1997). Additionally, heparan sulfate may also serve as counterligand for leukocyte adhesion molecules L-selectin and integrin Mac-1 (Diamond *et al*, 1995; Norgard-Sumnicht *et al*, 1993). In this view, chemokines create solid-phase rather than fluid chemotactic gradients, acting through a process termed haptotaxis. Importantly, MIP-1β and RANTES retain their chemotactic activities when bound to the extracellular matrix components (Gilat *et al*, 1994). Further, Pachter and colleagues documented high-affinity specific binding sites for beta chemokines on the abluminal surface of cerebrovascular endothelial cells (Addjelkovic *et al*, 1999).

Extravasation and parenchymal invasion are regulated within the CNS in a highly distinctive fashion (Figure 1). First, there are several ways that chemokine expression can be initiated in proximity to the elements of the BBB (Figure 1A). In immune-mediated inflammation, leukocytes in the CNS perivascular space can promote recruitment of additional inflammatory cells from the blood by secreting inflammatory cytokines like TNF-α and IL-1β that stimulate endothelial adhesion molecule expression and augment BBB leakage (Sharief *et al*, 1992).

Post-traumatic inflammation obviously produces direct focal impact on cellular elements of the BBB and acutely leads to chemokine production by activated endothelial elements or associated vessel-wall cells. Subsequently (days to weeks later), by unknown mechanisms, BBB function is compromised. Both neurohumoral signals and alterations in saturable transport systems have been implicated in delayed and widespread BBB decompensation after mechanical trauma.

Ischemic injury to the nervous system also directly damages the vascular bed, producing signals that elicit chemokine production. In all cases, the elaboration of chemokines, and upregulation of adhesion molecules of the immunoglobulin and selectin superfamilies on endothelial cells, appears to occur in close temporal relation. The elements are therefore established for local recruitment of leukocytes across the BBB and establishment of a perivascular inflammatory infiltrate. Further, current evidence indicates that signaling consequent to leukocyte transmigration probably leads directly to BBB disruption (Bolton *et al*, 1998).

Once localized in the perivascular space (Figure 1B), activated inflammatory cells may secrete chemokines like MIP-1α and RANTES that can induce extravasation of additional blood-borne inflammatory cells (Glabinski *et al*, 1996; Miyagishi *et al*, 1997). When present in abundance, inflammatory cells in CNS perivascular spaces develop many of the characteristics of lymphoid organs. Among these attributes are the high-level and mutually reinforcing expression of inflammatory cytokines including IFN-γ, TNF-α, IL-1 and IL-6. These constituents promote chemokine expression by surrounding glia, principally astrocytes. Invading leukocytes forming perivascular cuffs receive activating signals from these glial-derived chemokines and may further penetrate into the CNS parenchyma under the influence of chemokine

gradients (Figure 1C). Parenchymal astrocytes were shown to express MCP-1, IP-10 and GRO-α (Ransohoff *et al*, 1993; Tani *et al*, 1996a). These sequential alterations in BBB structure and function typify chronic inflammatory processes of the CNS, such as MS. Understanding their molecular details holds significant promise for the design of novel therapeutics.

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