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Roles of Chemokines and Their Receptors in Neuroinflammation

Andrzej R. Glabinski and Richard M. Ransohoff

1. CHEMOKINES: OVERVIEW

Chemokines are chemoattractant cytokines that stimulate directional migration of inflammatory cell in vitro and in vivo. Because of this, chemokines can be included into a large group of cytokines involved in the pathogenesis of inflammatory processes. All chemokines were identified within the last 20 yr and our knowledge about their roles in biology is rapidly growing. At present, an enormous amount of literature about chemokines and chemokine receptors is published each year.

There are now more than 50 different chemokines described in the literature. They are virtually all 8- to 10-kDa proteins with 20–70% homology in amino acid sequence. Chemokines are divided according to their structure into four main subfamilies: XCL, CCL, CXCL, and CX3CL. The criterion is the presence or absence of intervening amino acids between the first two cysteines near the N-terminus. If these cysteines are adjacent, the chemokine belongs to the CCL subfamily. The presence of one or three separating amino acids assigns the chemokine to the CXCL or CX3CL subfamily. The XCL subfamily possesses only one cysteine at the N-terminus. The CX3CL subfamily comprises only one chemokine: fractalkine. The XCL subfamily includes two chemokines: XCL1 (lymphotactin) and XCL2 (SCM1). The two other chemokine subfamilies, CXCL and CCL, are much larger and can be further subdivided. The CXCL family consists of at least 16 members, CCL is even larger—at least 28 members identified to date. The criterion for further division of the CXCL subfamily is the presence of the ELR motif (glutamate–leucine–arginine) near the N-terminus. This subdivision also has functional significance. Chemokines with the ELR motif attract neutrophils, whereas non-ELR CXC chemokines attract predominantly mononuclear inflammatory cells: monocytes and lymphocytes. CC chemokines can also be subdivided further into monocyte chemoattractant proteins (MCP-1–5) and others *(1)*.

Originally described as chemoattractant factors, chemokines turned out to be involved also in a large diversity of other physiological and pathological processes. They can not only guide leukocytes to inflammatory sites but also activate target cells at sites of injury. ELR-positive CXC chemokines (interleukin [IL]-8, GRO possess angiogenic activity, whereas ELR-negative interferon-inducible protein [IP-10], Mig) are angiostatic. Several chemokines may also induce smooth-muscle proliferation and induce cytokine production in lymphocytes *(2)*.

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2. CHEMOKINE RECEPTORS

Chemokines influence their target cells through chemokine receptors, which belong to seven transmembrane domain receptors signaling through the G-protein system. The homology between chemokine receptors is between 25% and 80% *(3)*. There are 11 chemokine receptors described so far for CC chemokines (CCR1-11), 6 for CXC chemokines (CXCR1-6), and 1 for each CX3C and C chemokine (XCR1 and CX3CR1, respectively). The interactions between chemokines and their receptors are complex and significant redundancy in this system is observed. It is frequently observed that multiple chemokines can bind to single chemokine receptor and that several chemokine receptors can respond to an individual chemokine ligand. Several chemokine receptors provide exceptions to this rule and are "monogamous." In general, chemokine receptors do not respond to ligands from distinct subfamilies. These properties resulted in the division of chemokine receptors into four main functional groups: shared, specific, promiscuous, and viral *(4)*. The examples of specific receptors are CXCR5 (ligand BCA-1), CCR6 (ligand MIP-3a), and CCR9 (ligand TECK). The cardinal example of a promiscuous chemokine receptor is Duffy antigen receptor for chemokines (DARC), which is expressed mainly on erythrocytes and postcapillary venules. Because it does not signal and is present abundantly on circulating erythrocytes, it was suggested that this receptor may serve as a "sink for chemokines," eliminating excess and promoting maintenance of chemokine gradient *(5)*. The last group of chemokine receptors are virus-encoded receptors. Their biological role(s) is not known. The most plausible hypothesis is that viruses "pirated" chemokine receptors to degrade host defenses during infection. In addition to virus-encoded chemokine receptors, some viruses also encode chemokine homologs and chemokine-binding proteins, presumably to achieve the same goals during infection *(6)*.

Chemokine receptors are present mainly on blood inflammatory cells (leukocytes). Some of them may be constitutively expressed like CCR2 on monocytes; others (like CCR5 on lymphocytes) have to be upregulated by inflammatory stimuli (e.g., IL-2) *(7)*. It was also recently shown that the division of acivated T-helper lymphocytes (Th) cells into pro-inflammatory Th1 and anti-inflammatory Th2 cells is also reflected by the expression of a different spectrum of chemokine receptors. Th1 cells express mainly CCR1, CCR5, and CXCR3 receptors, whereas Th2 predominantly CCR3, CCR4, and CCR8 *(8)*. Our knowledge about chemokine receptors increased significantly after the discovery that the human immunodeficiency virus (HIV) uses CCR5, CXCR4, and many other chemokine receptors as coreceptors for invasion of T-cells and monocytes.

Chemokine binding to chemokine receptors initiates upregulation of inositol triphosphate and intracellular calcium flux. Moreover, the activation of Ras and Rho families is induced. The Rho family plays a role in the formation of pseudopods involved in directional migration of inflammatory cells *(9)*.

3. CHEMOKINES AND LEUKOCYTE EXTRAVASATION

Chemokines are produced at tissue sites of inflammation by parenchymal cells that thereby induce the migration of inflammatory cells from the blood. Moreover, chemokines are produced by migrating leukocytes, thus augmenting the inflammatory process. The extravasation of leukocytes and their accumulation in an inflamed region is a

complicated and multistep process. It is initiated by adhesion molecules (i.e., selectins expressed on endothelium and leukocytes that interact with their carbohydrate receptors). This interaction causes leukocyte "rolling" on endothelium. At this stage, another group of adhesion molecules, integrins, initiate firm attachement of leukocytes to endothelium and their exposure to chemokine gradient. This signal stimulates transmigration of inflammatory cells from the vessel lumen to inflamed tissue. Chemokines play an important role during the extravasation step of this process, but they are also required to activate integrins and initiate leukocyte arrest and, in this way, accelerate the process of transmigration. Expression of specific sets of chemokines in an inflammatory region is responsible for the cellular composition of inflammatory foci.

The inflammatory process in the central nervous system (CNS) has unique features not seen in the periphery. The most important difference is the presence of the blood-brain barrier (BBB), which is composed of the nonfenestrated cerebrovascular endothelium sealed by tight junctions. Inflammatory cells migrating to the CNS must first penetrate the BBB and accumulate in perivascular/subarachnoid space *(10)*. Under physiological conditions, only activated T-cells penetrate this barrier during the process of immunological surveillance of the CNS *(11)*. Inflammation of immunological origin starts when patrolling T-cells encounter cognate antigen within the CNS parivascular space *(12)*. As a result, proinflammatory cytokines are produced by both T-cells and perivascular macrophages, stimulating CNS parenchymal cells to express chemokines (*see* Section 4). Astrocyte-derived chemokines may influence the BBB endothelium and attract antigen-nonspecific inflammatory cells to the nascent site of inflammation *(13)*. Inflammatory responses in the CNS also result from diverse other types of injury, including infection and mechanical, physical, chemical, and ischemic damage. Regardless of its origin, this response is usually characterized by chemokine overexpression (*see* Section 5).

4. EXPRESSION OF CHEMOKINES BY CNS CELLS IN VITRO

Although inflammatory leukocytes are the principal producers of chemokines and bearers of their receptors, cells of neural origin are also able to express chemokines and chemokine receptors. Initial studies showed that human glioma cell lines produce MCP-1 and IL-8 *(14,15)*. Cultured astrocytes stimulated with tumor necrosis factor-α (TNF-α) and transforming growth factor (TGF-β) express MCP-1 at both mRNA and protein levels *(16)* and astrocytoma cells stimulated with interferon-γ (IFN-γ) produce MCP-1 *(17)*. Stimulated astrocytes are also able to express monocyte inflammatory protein (MIP)-1α, MIP-1β, RANTES, and IP-10 *(18–20)*. Infection of cultured astrocytes with paramyxovirus NDV stimulates expression of IP-10 and RANTES *(21)*, and HIV-1 infection stimulates expression of IL-8 and IP-10 in affected astrocytes *(22)*. Infection of cultured human astrocytes with neurotropic coronavirus OC43 leads to increased expression of cytokines IL-6 and TNF-α, as well as chemokine MCP-1 *(23)*.

Microglial cells (especially after stimulation) have been also shown to be potent sources of some chemokines. After stimulation with IL-6 and colony-stimulating factor-1 (CSF-1) brain macrophages express MCP-1 *(24)*. Other inflammatory cytokines like TNF-α and IL-1β and lipopolysaccharide (LPS) may stimulate cultured microglia to produce MCP-1, MIP-1α, and MIP-1β *(25)*, IL-8 *(26)*, and RANTES *(27)*. It has been also shown that some infectious agents may stimulate overexpression of chemokines by microglia. For

example, the simian immunodeficiency (SIV) virus *(28)* and cryptococcal polysaccharide *(29)* can induce expression of IL-8 in cultured microglia.

Cultured brain endothelial cells can express MCP-1 spontaneously and this expression increases after stimulation with TNF-α *(30)*. It has been recently shown that cultured human cerebromicrovascular endothelial cells are able to express genes for MCP-1 and IL-8 when stimulated by hypoxic astrocytes, mediated by IL-1β *(31)*. Brain microvascular endothelial cells may express CXCR2 as well *(32)*. Parasitic infection of cultured brain endothelium stimulates expression of IL-8 *(33)*. Mixed human brain cell cultures stimulated with TNF-α expressed RANTES and MIP-1β *(34)*.

Chemokine receptors have also been found to be expressed on CNS cells in vitro and in vivo. Numerous studies showed that cultured astrocytes can express CXCR4, CX3CR1, CCR1, CCR10, and CCR11 *(35–37)*. In our studies, TGF-β1, but not IFN-γ, TNF-α, and LPS was able to stimulate primary mouse astrocytes to upregulate selectively CCR1 in vitro *(38)*. In the same model, TNF-α was a potent supressor of CXCR4 expression at the mRNA and protein levels *(39)*.

Microglia can express CCR3, CCR5, CXCR4, and CX3CR1 in vitro *(36,40,41)*. Cultured human neurons were shown to express CCR1, CCR5, CXCR2, and CXCR4 *(42)*. Another group showed that cultured human fetal neurons and the human neuronal cell line NT2.N can express CCR2, CXCR2, CXCR3 and CXCR4 at the mRNA and protein levels. Additionally, it has been shown in those studies that NT2.N neurons may produce chemokine MCP-1 *(43)*.

5. GENETICALLY PROGRAMMED OVEREXPRESSION OF CHEMOKINES IN CNS IN VIVO

Studies on chemokine expression under the control of CNS-specific promoters showed accumulation of appropriate subsets of leukocytes in this organ. These observations indicate that chemokines are potent inducers of selective recruitment of leukocyte subpopulations from the blood to the CNS in vivo. Transgenic mice expressing MCP-1 under control of the oligodendrocyte-specific MBP promoter exhibited selective monocyte accumulation in CNS perivascular spaces *(44)*. Intraperitoneal injection with LPS augmented this accumulation. Despite massive inflammatory infiltrates, transgenic mice did not show any evident neurological and behavioral deficits *(44)*. In another study, transgenic mice expressing chemokine KC in oligodendrocytes massive accumulation of neutrophils was found. The peak of this expression was observed between 2 and 3 wk of age in perivascular, meningeal, and parenchymal sites of CNS tissue *(45)*. Those mice developed delayed (beginning from 40 d of age) neurological syndrome of postural instability and rigidity. Neuropathological analysis showed BBB disruption and microglial activation *(45)*.

Those observations suggested that chemokines may selectively recruit specific subpopulations of inflammatory cells to the CNS and that this chemokine-driven recruitment is not invariably linked to leukocyte activation.

6. CHEMOKINES IN INFECTIOUS NEUROINFLAMMATION

Chemokines are important players in the formation of the inflammatory response of an organism to infectious challenge. Although neuroinflammation is characterized

by certain unique features (when compared to inflammation in other organs), other essential characteristics of inflammatory responses are conserved. One conserved mechanism is localized production of chemoattractant agents at sites of inflammation. In an early study analyzing chemokine involvement in the pathogenesis of experimental pneumococcal meningitis, Saukkonen and co-workers observed that intracisternal administration of antibodies blocking MIP-1α, MIP-1β, and MIP-2 during induction of the diseases delayed the onset of inflammation *(46)*. In brains from mice with encephalomyelitis induced by *Listeria*, expression of genes for MIP-1α, MIP-1β, and MIP-2 was also detected *(47)*. This expression was mainly localized in neutrophils accumulating in lateral and third ventricles starting from 12 h after disease induction. The highest level of MIP-1α and MIP-2 in corresponding cerebrospinal fluid (CSF) was found by enzyme-linked immunosorbent assay (ELISA) at 48–72 h postinfection *(47)*.

Many studies analyzed the level of chemokines in the CSF from patients with meningitis of different origins. It has been reported that there is a correlation between IL-8 and GRO-α levels and granulocyte counts in the CSF from patients with bacterial meningitis and between MCP-1 CSF levels and mononuclear cell counts in CSF from patients with nonbacterial meningitis *(48)*. Others observed a correlation between IL-8 concentration in CSF and neutrophil counts in patients with nonpyogenic meningitis *(49)*. In patients with pneumococcal, meningococcal, and *Haemophilus influenzae* bacterial meningitis, MCP-1, IL-8, and GRO-α, as well as also levels of MIP-1α and MIP-1β were elevated in the CSF *(50)*. This observation was extended by studies showing diminished migration of neutrophils after addition to the CSF of anti-IL-8 and anti-GRO-α antibodies. Migration of mononuclear cells was reduced in the same system by anti-MIP-1α, anti-MIP-1β, and anti-MCP antibodies *(50)*. A recent study analyzing the development of experimental brain abscess after embolization of *Staphalococcus aureus* beads showed involvement of chemokines in that process. Increased expression of neutrophil chemoattractant KC was detected 24 h after infection, whereas MCP-1 and MIP-1α were overexpressed in the brain within 24 h after bacterial exposure *(51)*.

Viral infections of the CNS parenchyma were also shown to be connected with increased chemokine levels in the CSF. In encephalitis caused by SIV, a primate model of human AIDS encephalitis, increased expression of MIP-1α, MIP-1β, MCP-1, MCP-3, RANTES, and IP-10 was detected *(52)*. Lymphocytic choriomeningitis infection led to increased expression of genes for MCP-1, MIP-1β, RANTES, IP-10, and MCP-3 in the brains of infected mice by 3 d after infection. A later increased expression of C-10, MIP-2, MIP-1α and lymphotactin was observed *(53)*. In encephalomyelitis caused by mouse hepatitis virus (MHV), increased expression of MIP-1α, MIP-2, IP-10, MCP-1, and RANTES was detected in the infected brain and spinal cord. Astrocytes expressed IP-10 in that model *(54)*. It has been proposed recently that Mig contributes significantly to the clearance of MHV CNS infection, as mice treated with anti-Mig antisera had much more severe disease. In the treated group, accumulation of CD4+ and CD8+ T-cells and expression of IFN-γ in the brain were significantly decreased *(55)*.

Increased expression of MIP-1α, MIP-1β, IP-10, MCP-1, and RANTES was present in murine brain during fatal hemorrhagic encephalopathy induced by infection with mouse adenovirus type-1 (MAV-1) *(56)*. The same infection caused increased upregulation of chemokine receptors CCR1-5 in BALB/c and C57BL/6 mice *(57)*. In dogs infected by canine distemper virus (CDV), an increased level of IL-8 was observed in

the CSF *(58)*. During meningoencephalitis induced by infection with the Borna disease virus (BDV), astrocytes were shown to express mRNA for IP-10 *(59)*. Theiler's virus model of multiple sclerosis (MS) was characterized by biphasic overexpression of chemokines IP-10, MCP-1, and RANTES, first during the acute inflammatory stage of the disease and, later, during demyelinating stage of infection *(60)*.

In brains from patients with HIV encephalitis, MCP-1, MIP-1α, and RANTES were detected on macrophages *(61)*. Moreover, in brains from patients with HIV-associated dementia, in which monocytic infiltration of the CNS is present, increased levels of MCP-1 were detected in brains and CSF *(62)*. The human T-cell lymphotrophic virus type-1 (HTLV-1) virus causes chronic progressive myelopathy with neurological and pathological features similar to progressive MS. Expression of MCP-1 has been observed in spinal cord lesions present in that disease *(63)*. Moreover, HTLV-1 -specific T-cell clones from patients with this myelopathy may express chemokines MIP-1α and MIP-1β *(64)*.

Additional information showing involvement of chemokines in infectious neuroinflammation was obtained by studying mice with disrupted chemokine and chemokine receptor genes. MIP-1α knockout mice infected with neurotropic fungus *Cryptococcus neoformans* had decreased leukocyte recruitment to the brain and impaired cryptococcal clearance from the brain *(65)*. In the CSF from patients with *Cryptococcal* meningitis, an increased level of IL-8 was found *(29)*.

7. CHEMOKINES IN IMMUNE-MEDIATED NEUROINFLAMMATION

The best characterized experimental neuroinflammation of immunological origin is experimental autoimmune encephalomyelitis (EAE). This disease can be induced in susceptible strains of laboratory animals (mice, rats, guinea pigs, monkeys) by immunization with CNS myelin protein antigens like myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG). EAE is an example of autoimmune inflammation of the CNS and is characterized by the presence of disseminated inflammatory "cuffs" around microvessels of the brain and spinal cord. EAE is considered to be a useful animal model of certain aspects of the human demyelinating disease MS. Both diseases share similar pathological features, although the autoimmune origin of MS has not been rigorously proven yet.

Initial descriptive reports showed that expression of some chemokines (MCP-1, IP-10) occurs during early stages of EAE *(66,67)* and those chemokines are expressed by astrocytes in the vicinity of inflammatory lesions *(67)*. Later, the expression of additional chemokines (MIP-1α, MIP-1β, RANTES, KC, MCP-3, TCA-3, fractalkine, MCP-5) was detected during acute EAE *(68–70)*. Our results showed that chemokine expression by parenchymal astrocytes during EAE do not initiate, but amplify, the ongoing CNS inflammatory process *(13)*. A correlation among MIP-1α, RANTES, and GRO-α expression and intensity of CNS inflammation was also reported *(71)*. A functional study found that the blockade of MIP-1α expression by antibody prevented the appearance of passively transferred acute EAE *(72)*. However, mice with knockout of MIP-1α and its receptor CCR5 were fully susceptible to MOG-induced EAE, showing the complexity of this subject *(73)*. Lately, in a variant of EAE in BALB/c mice characterized by pronounced neutrophil accumulation, increased expression of MIP-2 (chemokine-attracting neutrophils) as well as MIP-1α and MCP-1 was reported. Astrocytes were the main cellular source of MIP-2 and MIP-1α; infiltrating neutrophils expressed MIP-1α and MCP-1 *(74)*.

In chronic relapsing EAE (ChREAE), characterized by the spontaneous appearance of clinical relapses of the disease, we observed overexpression of chemokines MCP-1, IP10, GRO-α, MIP-1α, and RANTES concomittant with relapse. Astrocytes were the producers of MCP-1, IP-10, and GRO-α, whereas infiltrating inflammatory cells expressed RANTES and MIP-1α *(75)*. Anti-MCP-1, but not anti-MIP-1α, antibody was shown to significantly reduce the severity of relapses in ChREAE *(76)*. Recently, mice lacking the MCP-1 gene were shown to be markedly resistant to EAE induction and showed impaired recruitment of macrophages to the CNS *(77)*. The expression of chemokines within the CNS parenchyma during EAE is probably driven by inflammatory cytokines produced by migrating inflammatory cells as supported by observations in IFN-γ knockout mice (GKO) with EAE. In that model, we observed selectively diminished IP-10 expression, whereas non-IFN-γ-dependent chemokines, MCP-1 and GRO-α, were overexpressed *(78)*.

In addition to chemokines, CNS expression of chemokine receptors CXCR2, CXCR3, CXCR4, CX3CR1, CCR2, CCR5, and CCR8 was increased during EAE *(41,79)*. In ChREAE, the expression of CXCR2 and CXCR4 correlated with the appearance of new relapses *(80)*. Protection from EAE induced with altered peptide ligand (APL) was shown to reduce levels of several chemokines and also CXCR2, CXCR3, CCR1, CCR5, and CCR8 *(79)*. CCR6 and its ligand MIP-3a, a potent attractant of dendritic cells (DCs), were both upregulated in the CNS during EAE. In those studies, a prominent infiltration of mature DCs in the spinal cord of mice with acute and chronic EAE was described *(81)*. Two recent publications showed that CCR2 plays a necessary role in the pathogenesis of EAE. Mice with CCR2 knockout did not develop clinical EAE and failed to accumulate mononuclear inflammatory cells in the CNS. Moreover, they failed to upregulate RANTES, MCP-1, IP-10, CCR1, CCR2, and CCR5 during EAE *(82,83)*.

Many reports have been published lately addressing chemokine expression in MS. Reassuringly, these results resemble those obtained earlier in EAE. Hvas and co-workers detected RANTES by *in situ* hybridization in perivascular inflammatory cells *(84)*. In another study, MCP-1 was localized in astrocytes and inflammatory cells and MIP-1α and MIP-1β in inflammatory cells in active MS plaques *(85)*. Reactive astrocytes and inflammatory cells were also shown to express MCP-1, MCP-2, and MCP-3 in active MS lesions by others *(86)*. Recently, our group reported increased levels of IP-10, Mig, and RANTES in the CSF from patients with MS relapse *(87)*. The receptor for IP-10 and Mig (CXCR3) was detected on CSF cells, as well as lymphocytes in perivascular inflammatory cuffs; the receptor for RANTES (CCR5) was present on lymphocytes, macrophages, and microglial cells in active MS lesions *(87)*. Compatible results were published by others *(88)*. Analysis of chemotactic activity of T-cells from MS patients showed increased migratory rate toward chemokines RANTES and MIP-1α. This aberrant migration could be diminished by anti-CCR5 antibodies *(89)*. Moreover CCR2 and, to a smaller extent, CCR3 were detected in MS brains on CNS-infiltrating lymphocytes as well as on macrophages and microglia. Ligands for those receptors, MCP-1 and MCP-3, were localized around inflammatory foci *(85)*. The same group reported Mig, IP-10, and CXCR3 expression in actively demyelinating lesions by macrophages and reactive astrocytes in periplaque CNS tissue *(90)*. Treatment of remitting–relapsing MS with IFN-β reduced RANTES production in sera- and blood-adherent mononuclear cells both in relapse and in remission *(91)*.

8. CHEMOKINES IN TRAUMA TO THE NERVOUS SYSTEM

Several types of physical CNS injury have been shown to be followed by increased expression of chemokines. MCP-1 overexpression was observed after mechanical penetrating injury to the brain *(92,93)*. Astrocytes in the vicinity of the injury site expressed MCP-1 as early as a few hours after trauma *(93)*. Expression of other chemokines studied in that model was not increased. We observed a strict correlation between MCP-1 expression and the intensity of the inflammatory reaction in the brain. Out of four different injury models studied, the paradigm with the lowest intensity of inflammatory reaction (neonatal stab injury model) was typified by the lowest MCP-1 expression *(93)*. Other investigators showed increased expression of RANTES and MIP-1β 24 h after stab injury to the brain *(94)*. Immunohistochemistry localized MIP-1β in reactive astrocytes and macrophages at the site of injury, whereas RANTES was diffusely expressed in surrounding necrotic tissue *(94)*. Augmentation of mechanical cortical injury with LPS led to increased expression of several chemokines: MCP-1, MIP-1α, MIP-1β, RANTES, IP-10, and KC *(95)*. Antisense oligodeoxynucleotides that suppress MCP-1 protein expression diminished the accumulation of macrophages at the site of stab injury to the rat brain *(96)*. In a model of mechanical injury to the spinal cord, the expression of MIP-1α and MIP-1β was observed 1 d after injury diffusely in gray matter, later being present in inflammatory cells at the site of injury *(97)*. In a precisely calibrated contusion injury to the spinal cord, increased expression of other chemokines was also reported. MCP-1 >>> MCP-5 = GRO-α = IP-10 = MIP-3α were expressed within hours after injury and pre-ceded influx of inflammatory cells to the site of injury *(98)*.

Another type of injury, cryolesion of the cerebral cortex, induced increased expression of MCP-1, with a peak at 6 h after trauma. Another chemokine analyzed in that model, IP-10, was not overexpressed *(99)*. During chemical injury to the CNS induced by triethyltin (TET) overexpression of MIP-1α was detected *(100)*. It has been reported recently that MCP-1 may be an important mediator of acute excitotoxic injury induced by *N*-methyl-D-aspartate in the neonatal rat brain *(101)*. In the CSF from patients with severe brain trauma, the IL-8 concentration was significantly elevated. There was a clear correlation between the CSF IL-8 level and BBB disruption measured by the CSF/serum albumin ratio *(102)*.

9. CHEMOKINES IN ISCHEMIC INJURY TO THE NERVOUS SYSTEM

Several studies have demonstrated that one consequence of CNS ischemia is increased expression of chemokines. Early experiments reported increased expression of MCP-1 and MIP-1α 6 h after onset of brain ischemia *(103)*. In that study, endothelial cells and macrophages expressed MCP-1, whereas MIP-1α was described in astrocytic cells *(103)*. In another study, experimental middle cerebral artery occlusion (MCAO) also induced increased expression of MCP-1 beginning 6 h after injury *(104)*. Astrocytes provided the main cellular source of MCP-1 after MCAO; later (after 4 d) MCP-1 was expressed predominantly by macrophages and microglia at the ischemic area *(105)*. Overexpression of MIP-1α was detected in microglia localized in injured brain region after 4–6 h of ischemia *(106)*. In the rat model of MCAO, CXC chemokine cytokine-induced neutrophil chemoattractant (CINC) was overexpressed after 12 h of ischemia *(107)*. In the same model, increased expression of CXCR3 was observed and correlated with leukocyte

accumulation after focal brain ischemia *(108)*. In a neonatal model of brain hypoxia–ischemia, the peak of MCP-1 expression was detected at 8–24 h after the onset of ischemia and this expression returned to basal levels by 48 h *(109)*.

Pronounced reperfusion after focal brain ischemia may lead to additional brain damage and is usually a result of the accumulation of neutrophils. Chemokines attracting neutrophils like IL-8 and CINC were shown to be upregulated after brain reperfusion *(110)*. Blocking IL-8 with the antibody significantly reduced the size of infarcted brain tissue *(110)*. In a rat forebrain reperfusion injury model, MCP-1 expression was detected at the transcript level as early as 1 h after reperfusion *(111)*.

In patients with subarachnoid hemorrhage, MCP-1 and IL-8 levels in the CSF were significantly increased compared with patients with unruptured aneurysms *(112)*.

10. CHEMOKINES IN NEURODEGENERATION

In the course of neurodegenerative disorders, the BBB remains intact and migration of inflammatory cells from the blood to the CNS is not observed. Therefore, inflammatory cells detected during chronic neurodegenerative pathologies are CNS macrophages/microglia. Chemokine and chemokine receptor expression was extensively studied in Alzheimer's disease (AD), the most common nerodegenerative disease causing dementia. It has been shown that CXCR2 immunostaining is present in senile plaques and correlates with APP expression *(113,114)*. Additionally, increased expression of CCR3 and CCR5 in reactive microglia of AD brains was reported. In that study, MIP-1β was detected in AD predominantly in reactive astrocytes *(115)*. The same group reported overexpression of IP-10 and its receptor CXCR3 in astrocytes in AD brains *(116)*. Other authors observed increased expression of MCP-1 in mature senile plaques and reactive microglia from patients with AD *(117)*. Additional information from in vitro studies confirms the possible involvement of chemokines and their receptors in AD pathogenesis. β-Amyloid was shown to stimulate cultured brain macrophages to produce MCP-1 *(118)* and astrocytoma cells for production of IL-8 *(119)*. Interestingly, RANTES was shown to be neuroprotective when added to neuronal cultures exposed to toxic fragment of β-amyloid peptide *(120)*.

In an experimental model of thalamic retrograde neurodegeneration induced by damage to the cerebral cortex, rapid overexpression of MCP-1 in a thalamus ipsilateral to injury was observed. This expression was localized by *in situ* hybridization to glial cells of the lateral geniculate nucleus *(121)*.

11. CHEMOKINES IN PERIPHERAL NERVOUS SYSTEM PATHOLOGY

After axotomy in the peripheral nervous system, macrophages accumulate at the site of nerve transection. It has been hypothesized that this inflammatory reaction is the principal factor promoting regeneration of injured periperal nerve. It has been reported that expression of MCP-1 preceded recruitment of macrophages to the injury region and was localized by *in situ* hybridization in Schwann cells *(122)*. In a recent study analyzing chemokine expression in the experimental lesion of facial and hypoglossal nerves, MCP-1 was expressed by damaged neurons. Expression of RANTES and IP-10 as well as the MCP-1 receptor CCR2 was not elevated *(123)*. In a model of peripheral Wallerian degeneration induced in CCR2 knockout mice, a macrophage invasion after

sciatic nerve transection was significantly impaired. In sciatic axotomy of CCR5-deficient mice, this finding was not observed *(124)*.

An animal model of human Guillain–Barre polyneuropathy, experimental autoimmune neuritis (EAN) is characterized by the presence of mononuclear inflammatory cells (lymphocytes and macrophages) in affected nerves. It has been reported that MCP-1 expression increases shortly before clinical signs of this disease *(125)*. In another study in that model, the peak of MIP-1α and MIP-1β expression preceded maximum disease severity, whereas the maximum expression of MCP-1, RANTES, and IP-10 was present at the time of peak of the disease. RANTES expression was localized within invading lymphocytes, and IP-10 was detected mainly in perineurial endothelium *(126)*. In trigeminal ganglia from mice infected at least 5 d earlier with herpes simplex virus type I, increased expression of RANTES was found *(127)*.

12. CONCLUSIONS

The data described in this review show that chemokines play important roles in diverse nervous system pathologies. Originally described as chemotactic agents, chemokines have recently been found to be crucial factors in damage to nervous tissue, as key mediators of inflammatory responses. Chemokine involvement has been reported in neuroinfections, autoimmune pathologies, neurotrauma after mechanical, physical, or chemical injury, and ischemia. Rapidly accumulating information about the extra-inflammatory properties of chemokines has also impacted the neurosciences. Currently, chemokines and their receptors are considered to be important factors in neurodegenerative processes, nervous tissue development, and neuron–glia communication. One may expect that, in the near future, our knowledge in this area will expand further. However, even at present, chemokines can be considered important targets for new therapies, especially in neuroinflammatory conditions.

REFERENCES

1. Rossi, D. and Zlotnik, A. (2000) The biology of chemokines and their receptors. *Ann. Rev. Immunol.* **18,** 217–242.
2. Lukacs, N. W., Hogaboam, C., Campbell, E., and Kunkel, S. L. (1999) Chemokines: function, regulation and alteration of inflammatory responses. *Chem. Immunol.* **72,** 102–120.
3. Kelvin, D. J., Michiel, D. F., Johnston, J. A., Lloyd, A. R., Sprenger, H., Oppenheim, J. J., et al. (1993) Chemokines and serpentines: the molecular biology of chemokine receptors. *J. Leukocyte Biol.* **54,** 604–612.
4. Premack, B. A. and Schall, T. J. (1996) Chemokine receptors: gateways to inflammation and infection. *Nat. Med.* **2,** 1174–1178.
5. Murphy, P. M., Baggiolini, M., Charo, I. F., Hebert, C. A., Horuk, R., Matsushima, K., et al. (2000) International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol. Rev.* **52,** 145–176.
6. Lalani, A. S., Barrett, J. W., and McFadden, G. (2000) Modulating chemokines: more lessons from viruses. *Immunol. Today* **21,** 100–106.
7. Alkhatib, G., Combadiere, C., Broder, C. C., Feng, Y., Kennedy, P. E., Murphy, P. M., et al. (1996) CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* **272,** 1955–1958.
8. Sallusto, F., Lanzavecchia, A., and Mackay, C. R. (1998) Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. *Immunol. Today* **19,** 568–574.

9. Laudanna, C., Campbell, J. J., and Butcher, E. C. (1996) Role of Rho in chemoattractant-activated leukocyte adhesion through integrins. *Science* **271,** 981–983.
10. Glabinski, A. R. and Ransohoff, R. M. (1999) Sentries at the gate: chemokines and the blood-brain barrier. *J. Neurovirol.* **5,** 623–634.
11. Wekerle, H., Engelhardt, B., Risau, W., and Meyermann, R. (1991) Interaction of T lymphocytes with cerebral endothelial cells in vitro. *Brain Pathol.* **1,** 107–114.
12. Hickey, W. F. (1991) Migration of hematogenous cells through the blood-brain barrier and the initiation of CNS inflammation. *Brain Pathol.* **1,** 97–105.
13. Glabinski, A. R., Tani, M., Tuohy, V. K., Tuthill, R. J., and Ransohoff, R. M. (1995) Central nervous system chemokine mRNA accumulation follows initial leukocyte entry at the onset of acute murine experimental autoimmune encephalomyelitis. *Brain Behav. Immun.* **9,** 315–330.
14. Yoshimura, T., Robinson, E. A., Tanaka, S., Appella, E., Kuratsu, J., and Leonard, E. J. (1989) Purification and amino acid analysis of two human glioma-derived monocyte chemoattractants. *J. Exp. Med.* **169,** 1449–1459.
15. Morita, M., Kasahara, T., Mukaida, N., Matsushima, K., Nagashima, T., Nishizawa, M., et al. (1993) Induction and regulation of IL-8 and MCAF production in human brain tumor cell lines and brain tumor tissues. *Eur. Cytokine Network* **4,** 351–358.
16. Hurwitz, A. A., Lyman, W. D., and Berman, J. W. (1995) Tumor necrosis factor alpha and transforming growth factor beta upregulate astrocyte expression of monocyte chemoattractant protein-1. *J. Neuroimmunol.* **57,** 193–198.
17. Zhou, Z. H., Chaturvedi, P., Han, Y. L., Aras, S., Li, Y. S., Kolattukudy, P. E., et al. (1998) IFN-gamma induction of the human monocyte chemoattractant protein (hMCP)-1 gene in astrocytoma cells: functional interaction between an IFN-gamma-activated site and a GC-rich element. *J. Immunol.* **160,** 3908–3916.
18. Barna, B. P., Pettay, J., Barnett, G. H., Zhou, P., Iwasaki, K., and Estes, M. L. (1994) Regulation of monocyte chemoattractant protein-1 expression in adult human non-neoplastic astrocytes is sensitive to tumor necrosis factor (TNF) or antibody to the 55-kDa TNF receptor. *J. Neuroimmunol.* **50,** 101–107.
19. Majumder, S., Zhou, L. Z., and Ransohoff, R. M. (1996) Transcriptional regulation of chemokine gene expression in astrocytes. *J. Neurosci. Res.* **45,** 758–769.
20. Peterson, P. K., Hu, S., Salak-Johnson, J., Molitor, T. W., and Chao, C. C. (1997) Differential production of and migratory response to beta chemokines by human microglia and astrocytes. *J. Infect. Dis.* **175,** 478–481.
21. Fisher, S. N., Vanguri, P., Shin, H. S., and Shin, M. L. (1995) Regulatory mechanisms of MuRantes and CRG-2 chemokine gene induction in central nervous system glial cells by virus. *Brain Behav. Immun.* **9,** 331–344.
22. Kutsch, O., Oh, J., Nath, A., and Benveniste, E. N. (2000) Induction of the chemokines interleukin-8 and IP-10 by human immunodeficiency virus type 1 tat in astrocytes. *J. Virol.* **74,** 9214–9221.
23. Edwards, J. A., Denis, F., and Talbot, P. J. (2000) Activation of glial cells by human coronavirus OC43 infection. *J. Neuroimmunol.* **108,** 73–81.
24. Calvo, C. F., Yoshimura, T., Gelman, M., and Mallat, M. (1996) Production of monocyte chemotactic protein-1 by rat brain macrophages. *Eur. J. Neurosci.* **8,** 1725–1734.
25. McManus, C. M., Brosnan, C. F., and Berman, J. W. (1998) Cytokine induction of MIP-1 alpha and MIP-1 beta in human fetal microglia. *J. Immunol.* **160,** 1449–1455.
26. Ehrlich, L. C., Hu, S., Sheng, W. S., Sutton, R. L., Rockswold, G. L., Peterson, P. K., et al. (1998) Cytokine regulation of human microglial cell IL-8 production. *J. Immunol.* **160,** 1944–1948.
27. Kitai, R., Zhao, M., Zhang, N., Hua, L. L., and Lee, S. C. (2000) Role of MIP-1beta and RANTES in HIV-1 infection of microglia: inhibition of infection and induction by IFNbeta. *J. Neuroimmunol.* **110,** 230–239.

28. Sopper, S., Demuth, M., Stahl-Hennig, C., Hunsmann, G., Plesker, R., Coulibaly, C., et al. (1996) The effect of simian immunodeficiency virus infection in vitro and in vivo on the cytokine production of isolated microglia and peripheral macrophages from rhesus monkey. *Virology* **220,** 320–329.
29. Lipovsky, M. M., Gekker, G., Hu, S., Ehrlich, L. C., Hoepelman, A. I., and Peterson, P. K. (1998) Cryptococcal glucuronoxylomannan induces interleukin (IL)-8 production by human microglia but inhibits neutrophil migration toward IL-8. *J. Infect. Dis.* **177,** 260–263.
30. Zach, O., Bauer, H. C., Richter, K., Webersinke, G., Tontsch, S., and Bauer, H. (1997) Expression of a chemotactic cytokine (MCP-1) in cerebral capillary endothelial cells in vitro. *Endothelium* **5,** 143–153.
31. Zhang, W., Smith, C., Howlett, C., and Stanimirovic, D. (2000) Inflammatory activation of human brain endothelial cells by hypoxic astrocytes in vitro is mediated by IL-1beta. *J. Cereb. Blood Flow Metab.* **20,** 967–978.
32. Otto, V. I., Heinzel-Pleines, U. E., Gloor, S. M., Trentz, O., Kossmann, T., and Morganti-Kossmann, M. C. (2000) sICAM-1 and TNF-alpha induce MIP-2 with distinct kinetics in astrocytes and brain microvascular endothelial cells. *J. Neurosci. Res.* **60,** 733–742.
33. Bourdoulous, S., Bensaid, A., Martinez, D., Sheikboudou, C., Trap, I., Strosberg, A. D., et al. (1995) Infection of bovine brain microvessel endothelial cells with Cowdria ruminantium elicits IL-1 beta, -6, and -8 mRNA production and expression of an unusual MHC class II DQ alpha transcript. *J. Immunol.* **154,** 4032–4038.
34. Lokensgard, J. R., Gekker, G., Ehrlich, L. C., Hu, S., Chao, C. C., and Peterson, P. K. (1997) Proinflammatory cytokines inhibit HIV-1 (SF162) expression in acutely infected human brain cell cultures. *J. Immunol.* **158,** 2449–2455.
35. Heesen, M., Tanabe, S., Berman, M. A., Yoshizawa, I., Luo, Y., Kim, R. J., et al. (1996) Mouse astrocytes respond to the chemokines MCP-1 and KC, but reverse transcriptase–polymerase chain reaction does not detect mRNA for the KC or new MCP-1 receptor. *J. Neurosci. Res.* **45,** 382–391.
36. Tanabe, S., Heesen, M., Berman, M. A., Fischer, M. B., Yoshizawa, I., Luo, Y., et al. (1997) Murine astrocytes express a functional chemokine receptor. *J. Neurosci.* **17,** 6522–6528.
37. Dorf, M. E., Berman, M. A., Tanabe, S., Heesen, M., and Luo, Y. (2000) Astrocytes express functional chemokine receptors. *J. Neuroimmunol.* **111,** 109–121.
38. Han, Y., Wang, J., Zhou, Z., and Ransohoff, R. M. (2000) TGFbeta1 selectively up-regulates CCR1 expression in primary murine astrocytes. *Glia* **30,** 1–10.
39. Han, Y., Wang, J., He, T., and Ransohoff, R. M. (2001) TNF-alpha down-regulates CXCR4 expression in primary murine astrocytes. *Brain Res.* **888,** 1–10.
40. He, J., Chen, Y., Farzan, M., Choe, H., Ohagen, A., Gartner, S., et al. (1997) CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* **385,** 645–649.
41. Jiang, Y., Salafranca, M. N., Adhikari, S., Xia, Y., Feng, L., Sonntag, M. K., et al. (1998) Chemokine receptor expression in cultured glia and rat experimental allergic encephalomyelitis. *J. Neuroimmunol.* **86,** 1–12.
42. Hesselgesser, J., Halks-Miller, M., DelVecchio, V., Peiper, S. C., Hoxie, J., Kolson, D. L., et al. (1997) CD4-independent association between HIV-1 gp120 and CXCR4: functional chemokine receptors are expressed in human neurons. *Curr. Biol.* **7,** 112–121.
43. Coughlan, C. M., McManus, C. M., Sharron, M., Gao, Z., Murphy, D., Jaffer, S., et al. (2000) Expression of multiple functional chemokine receptors and monocyte chemoattractant protein-1 in human neurons. *Neuroscience* **97,** 591–600.
44. Fuentes, M. E., Durham, S. K., Swerdel, M. R., Lewin, A. C., Barton, D. S., Megill, J. R., et al. (1995) Controlled recruitment of monocytes and macrophages to specific organs through transgenic expression of monocyte chemoattractant protein-1. *J. Immunol.* **155,** 5769–5776.
45. Tani, M., Fuentes, M. E., Peterson, J. W., Trapp, B. D., Durham, S. K., Loy, J. K., et al. (1996) Neutrophil infiltration, glial reaction, and neurological disease in transgenic mice expressing the chemokine N51/KC in oligodendrocytes. *J. Clin. Invest.* **98,** 529–539.

46. Saukkonen, K., Sande, S., Cioffe, C., Wolpe, S., Sherry, B., Cerami, A., et al. (1990) The role of cytokines in the generation of inflammation and tissue damage in experimental gram-positive meningitis. *J. Exp. Med.* **171,** 439–448.
47. Seebach, J., Bartholdi, D., Frei, K., Spanaus, K. S., Ferrero, E., Widmer, U., et al. (1995) Experimental *Listeria* meningoencephalitis. Macrophage inflammatory protein-1 alpha and -2 are produced intrathecally and mediate chemotactic activity in cerebrospinal fluid of infected mice. *J. Immunol.* **155,** 4367–4375.
48. Sprenger, H., Rosler, A., Tonn, P., Braune, H. J., Huffmann, G., and Gemsa, D. (1996) Chemokines in the cerebrospinal fluid of patients with meningitis. *Clin. Immunol. Immunopathol.* **80,** 155–161.
49. Lopez-Cortes, L. F., Cruz-Ruiz, M., Gomez-Mateos, J., Viciana-Fernandez, P., Martinez-Marcos, F. J., and Pachon, J. (1995) Interleukin-8 in cerebrospinal fluid from patients with meningitis of different etiologies: its possible role as neutrophil chemotactic factor. *J. Infect. Dis.* **172,** 581–584.
50. Spanaus, K. S., Nadal, D., Pfister, H. W., Seebach, J., Widmer, U., Frei, K., et al. (1997) C-X-C and C-C chemokines are expressed in the cerebrospinal fluid in bacterial meningitis and mediate chemotactic activity on peripheral blood-derived polymorphonuclear and mononuclear cells in vitro. *J. Immunol.* **158,** 1956–1964.
51. Kielian, T. and Hickey, W. F. (2000) Proinflammatory cytokine, chemokine, and cellular adhesion molecule expression during the acute phase of experimental brain abscess development. *Am. J. Pathol.* **157,** 647–658.
52. Sasseville, V. G., Smith, M. M., Mackay, C. R., Pauley, D. R., Mansfield, K. G., Ringler, D. J., et al. (1996) Chemokine expression in simian immunodeficiency virus-induced AIDS encephalitis. *Am. J. Pathol.* **149,** 1459–1467.
53. Asensio, V. C. and Campbell, I. L. (1997) Chemokine gene expression in the brains of mice with lymphocytic choriomeningitis. *J. Virol.* **71,** 7832–7840.
54. Lane, T. E., Asensio, V. C., Yu, N., Paoletti, A. D., Campbell, I. L., and Buchmeier, M. J. (1998) Dynamic regulation of alpha- and beta-chemokine expression in the central nervous system during mouse hepatitis virus-induced demyelinating disease. *J. Immunol.* **160,** 970–978.
55. Liu, M. T., Armstrong, D., Hamilton, T. A., and Lane, T. E. (2001) Expression of Mig (monokine induced by interferon-gamma) is important in T lymphocyte recruitment and host defense following viral infection of the central nervous system. *J. Immunol.* **166,** 1790–1795.
56. Charles, P. C., Weber, K. S., Cipriani, B., and Brosnan, C. F. (1999) Cytokine, chemokine and chemokine receptor mRNA expression in different strains of normal mice: implications for establishment of a Th1/Th2 bias. *J. Neuroimmunol.* **100,** 64–73.
57. Charles, P. C., Chen, X., Horwitz, M. S., and Brosnan, C. F. (1999) Differential chemokine induction by the mouse adenovirus type-1 in the central nervous system of susceptible and resistant strains of mice. *J. Neurovirol.* **5,** 55–64.
58. Tipold, A., Moore, P., Zurbriggen, A., Burgener, I., Barben, G., and Vandevelde, M. (1999) Early T cell response in the central nervous system in canine distemper virus infection. *Acta Neuropathol. (Berl.)* **97,** 45–56.
59. Sauder, C., Hallensleben, W., Pagenstecher, A., Schneckenburger, S., Biro, L., Pertlik, D., et al. (2000) Chemokine gene expression in astrocytes of Borna disease virus-infected rats and mice in the absence of inflammation. *J. Virol.* **74,** 9267–9280.
60. Murray, P. D., Krivacic, K., Chernosky, A., Wei, T., Ransohoff, R. M., and Rodriguez, M. (2000) Biphasic and regionally-restricted chemokine expression in the central nervous system in the Theiler's virus model of multiple sclerosis. *J. Neurovirol.* **6(Suppl. 1),** S44–S52.
61. Sanders, V. J., Pittman, C. A., White, M. G., Wang, G., Wiley, C. A., and Achim, C. L. (1998) Chemokines and receptors in HIV encephalitis. *AIDS* **12,** 1021–1026.

62. Conant, K., Garzino-Demo, A., Nath, A., McArthur, J. C., Halliday, W., Power, C., et al. (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.
63. Umehara, F., Izumo, S., Takeya, M., Takahashi, K., Sato, E., and Osame, M. (1996) Expression of adhesion molecules and monocyte chemoattractant protein-1 (MCP-1) in the spinal cord lesions in HTLV-I-associated myelopathy. *Acta Neuropathol.* **91,** 343–350.
64. Biddison, W. E., Kubota, R., Kawanishi, T., Taub, D. D., Cruikshank, W. W., Center, D. M., et al. (1997) Human T cell leukemia virus type I (HTLV-I)-specific CD8+ CTL clones from patients with HTLV-I-associated neurologic disease secrete proinflammatory cytokines, chemokines, and matrix metalloproteinase. *J. Immunol.* **159,** 2018–2025.
65. Huffnagle, G. B. and McNeil, L. K. (1999) Dissemination of C. neoformans to the central nervous system: role of chemokines, Th1 immunity and leukocyte recruitment. *J. Neurovirol.* **5,** 76–81.
66. Hulkower, K., Brosnan, C. F., Aquino, D. A., Cammer, W., Kulshrestha, S., Guida, M. P., et al. (1993) Expression of CSF-1, c-fms, and MCP-1 in the central nervous system of rats with experimental allergic encephalomyelitis. *J. Immunol.* **150,** 2525–2533.
67. Ransohoff, R. M., Hamilton, T. A., Tani, M., Stoler, M. H., Shick, H. E., Major, J. A., et al. (1993) Astrocyte expression of mRNA encoding cytokines IP-10 and JE/MCP-1 in experimental autoimmune encephalomyelitis. *FASEB J.* **7,** 592–600.
68. Godiska, R., Chantry, D., Dietsch, G. N., and Gray, P. W. (1995) Chemokine expression in murine experimental allergic encephalomyelitis. *J. Neuroimmunol.* **58,** 167–176.
69. Pan, Y., Lloyd, C., Zhou, H., Dolich, S., Deeds, J., Gonzalo, J. A., et al. (1997) Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation. *Nature* **387,** 611–617.
70. Sun, D., Tani, M., Newman, T. A., Krivacic, K., Phillips, M., Chernosky, A., et al. (2000) Role of chemokines, neuronal projections, and the blood-brain barrier in the enhancement of cerebral EAE following focal brain damage. *J. Neuropathol. Exp. Neurol.* **59,** 1031–1043.
71. Glabinski, A. R., Tuohy, V. K., and Ransohoff, R. M. (1998) Expression of chemokines RANTES, MIP-1alpha and GRO-alpha correlates with inflammation in acute experimental autoimmune encephalomyelitis. *Neuroimmunomodulation* **5,** 166–171.
72. Karpus, W. J., Lukacs, N. W., McRae, B. L., Strieter, R. M., Kunkel, S. L., and Miller, S. D. (1995) An important role for the chemokine macrophage inflammatory protein-1 alpha in the pathogenesis of the T cell-mediated autoimmune disease, experimental autoimmune encephalomyelitis. *J. Immunol.* **155,** 5003–5010.
73. Tran, E. H., Kuziel, W. A., and Owens, T. (2000) Induction of experimental autoimmune encephalomyelitis in C57BL/6 mice deficient in either the chemokine macrophage inflammatory protein-1alpha or its CCR5 receptor. *Eur. J. Immunol.* **30,** 1410–1415.
74. Nygardas, P. T., Maatta, J. A., and Hinkkanen, A. E. (2000) Chemokine expression by central nervous system resident cells and infiltrating neutrophils during experimental autoimmune encephalomyelitis in the BALB/c mouse. *Eur. J. Immunol.* **30,** 1911–1918.
75. Glabinski, A. R., Tani, M., Strieter, R. M., Tuohy, V. K., and Ransohoff, R. M. (1997) Synchronous synthesis of alpha- and beta-chemokines by cells of diverse lineage in the central nervous system of mice with relapses of chronic experimental autoimmune encephalomyelitis. *Am. J. Pathol.* **150,** 617–630.
76. Karpus, W. J. and Kennedy, K. J. (1997) MIP-1alpha and MCP-1 differentially regulate acute and relapsing autoimmune encephalomyelitis as well as Th1/Th2 lymphocyte differentiation. *J. Leukocyte Biol.* **62,** 681–687.
77. Huang, D., Wang, J., Kivisakk, P., Rollins, B. J., and Ransohoff, R. M. (2001) Absence of monocyte chemoattractant protein 1 in mice leads to decreased local macrophage recruitment and antigen-specific T helper cell type 1 immune response in experimental autoimmune encephalomyelitis. *J. Exp. Med.* **193,** 713–725.

78. Glabinski, A. R., Krakowski, M., Han, Y., Owens, T., and Ransohoff, R. M. (1999) Chemokine expression in GKO mice (lacking interferon-gamma) with experimental autoimmune encephalomyelitis. *J. Neurovirol.* **5,** 95–101.
79. Fischer, F. R., Santambrogio, L., Luo, Y., Berman, M. A., Hancock, W. W., and Dorf, M. E. (2000) Modulation of experimental autoimmune encephalomyelitis: effect of altered peptide ligand on chemokine and chemokine receptor expression. *J. Neuroimmunol.* **110,** 195–208.
80. Glabinski, A. R., O'Bryant, S., Selmaj, K., and Ransohoff, R. M. (2000) CXC chemokine receptor expression during chronic relapsing experimental autoimmune encephalomyelitis. *Ann. NY Acad. Sci.* **917,** 135–144.
81. Serafini, B., Columba-Cabezas, S., Di Rosa, F., and Aloisi, F. (2000) Intracerebral recruitment and maturation of dendritic cells in the onset and progression of experimental autoimmune encephalomyelitis. *Am. J. Pathol.* **157,** 1991–2002.
82. Fife, B. T., Huffnagle, G. B., Kuziel, W. A., and Karpus, W. J. (2000) CC chemokine receptor 2 is critical for induction of experimental autoimmune encephalomyelitis. *J. Exp. Med.* **192,** 899–906.
83. Izikson, L., Klein, R. S., Charo, I. F., Weiner, H. L., and Luster, A. D. (2000) Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2. *J. Exp. Med.* **192,** 1075–1080.
84. Hvas, J., McLean, C., Justesen, J., Kannourakis, G., Steinman, L., Oksenberg, J. R., et al. (1997) Perivascular T cells express the pro-inflammatory chemokine RANTES mRNA in multiple sclerosis lesions. *Scand. J. Immunol.* **46,** 195–203.
85. Simpson, J. E., Newcombe, J., Cuzner, M. L., and Woodroofe, M. N. (1998) Expression of monocyte chemoattractant protein-1 and other beta-chemokines by resident glia and inflammatory cells in multiple sclerosis lesions. *J. Neuroimmunol.* **84,** 238–249.
86. McManus, C., Berman, J. W., Brett, F. M., Staunton, H., Farrell, M., and Brosnan, C. F. (1998) MCP-1, MCP-2 and MCP-3 expression in multiple sclerosis lesions: an immunohistochemical and in situ hybridization study. *J. Neuroimmunol.* **86,** 20–29.
87. Sorensen, T. L., Tani, M., Jensen, J., Pierce, V., Lucchinetti, C., Folcik, V. A., et al. (1999) Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J. Clin. Invest.* **103,** 807–815.
88. Balashov, K. E., Rottman, J. B., Weiner, H. L., and Hancock, W. W. (1999) CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. *Proc. Natl. Acad. Sci. USA* **96,** 6873–6878.
89. Zang, Y. C., Samanta, A. K., Halder, J. B., Hong, J., Tejada-Simon, M. V., Rivera, V. M., et al. (2000) Aberrant T cell migration toward RANTES and MIP-1 alpha in patients with multiple sclerosis. Overexpression of chemokine receptor CCR5. *Brain* **123,** 1874–1882.
90. Simpson, J. E., Newcombe, J., Cuzner, M. L., and Woodroofe, M. N. (2000) Expression of the interferon-gamma-inducible chemokines IP-10 and Mig and their receptor, CXCR3, in multiple sclerosis lesions. *Neuropathol. Appl. Neurobiol.* **26,** 133–142.
91. Iarlori, C., Reale, M., Lugaresi, A., De Luca, G., Bonanni, L., Di Iorio, A., et al. (2000) RANTES production and expression is reduced in relapsing-remitting multiple sclerosis patients treated with interferon-beta-1b. *J. Neuroimmunol.* **107,** 100–107.
92. Berman, J. W., Guida, M. P., Warren, J., Amat, J., and Brosnan, C. F. (1996) Localization of monocyte chemoattractant peptide-1 expression in the central nervous system in experimental autoimmune encephalomyelitis and trauma in the rat. *J. Immunol.* **156,** 3017–3023.
93. Glabinski, A. R., Balasingam, V., Tani, M., Kunkel, S. L., Strieter, R. M., Yong, V. W., et al. (1996) Chemokine monocyte chemoattractant protein-1 is expressed by astrocytes after mechanical injury to the brain. *J. Immunol.* **156,** 4363–4368.
94. Ghirnikar, R. S., Lee, Y. L., He, T. R., and Eng, L. F. (1996) Chemokine expression in rat stab wound brain injury. *J. Neurosci. Res.* **46,** 727–733.

95. Hausmann, E. H., Berman, N. E., Wang, Y. Y., Meara, J. B., Wood, G. W., and Klein, R. M. (1998) Selective chemokine mRNA expression following brain injury. *Brain Res.* **788,** 49–59.
96. Ghirnikar, R. S., Lee, Y. L., Li, J. D., and Eng, L. F. (1998) Chemokine inhibition in rat stab wound brain injury using antisense oligodeoxynucleotides. *Neurosci. Lett.* **247,** 21–24.
97. Bartholdi, D. and Schwab, M. E. (1997) Expression of pro-inflammatory cytokine and chemokine mRNA upon experimental spinal cord injury in mouse: an in situ hybridization study. *Eur. J. Neurosci.* **9,** 1422–1438.
98. McTigue, D. M., Tani, M., Krivacic, K., Chernosky, A., Kelner, G. S., Maciejewski, D., et al. (1998) Selective chemokine mRNA accumulation in the rat spinal cord after contusion injury. *J. Neurosci. Res.* **53,** 368–376.
99. Grzybicki, D., Moore, S. A., Schelper, R., Glabinski, A. R., Ransohoff, R. M., and Murphy, S. (1998) Expression of monocyte chemoattractant protein (MCP-1) and nitric oxide synthase-2 following cerebral trauma. *Acta Neuropathol. (Berl.)* **95,** 98–103.
100. Mehta, P. S., Bruccoleri, A., Brown, H. W., and Harry, G. J. (1998) Increase in brain stem cytokine mRNA levels as an early response to chemical-induced myelin edema. *J. Neuroimmunol.* **88,** 154–164.
101. Galasso, J. M., Liu, Y., Szaflarski, J., Warren, J. S., and Silverstein, F. S. (2000) Monocyte chemoattractant protein-1 is a mediator of acute excitotoxic injury in neonatal rat brain. *Neuroscience* **101,** 737–744.
102. Kossmann, T., Stahel, P. F., Lenzlinger, P. M., Redl, H., Dubs, R. W., Trentz, O., et al. (1997) Interleukin-8 released into the cerebrospinal fluid after brain injury is associated with blood-brain barrier dysfunction and nerve growth factor production. *J. Cereb. Blood Flow Metab.* **17,** 280–289.
103. Kim, J. S. (1996) Cytokines and adhesion molecules in stroke and related diseases. *J. Neurol. Sci.* **137,** 69–78.
104. Wang, X., Yue, T. L., Barone, F. C., and Feuerstein, G. Z. (1995) Monocyte chemoattractant protein-1 messenger RNA expression in rat ischemic cortex. *Stroke* **26,** 661–665; discussion 665–666.
105. Gourmala, N. G., Buttini, M., Limonta, S., Sauter, A., and Boddeke, H. W. (1997) Differential and time-dependent expression of monocyte chemoattractant protein-1 mRNA by astrocytes and macrophages in rat brain: effects of ischemia and peripheral lipopolysaccharide administration. *J. Neuroimmunol.* **74,** 35–44.
106. Takami, S., Nishikawa, H., Minami, M., Nishiyori, A., Sato, M., Akaike, A., et al. (1997) Induction of macrophage inflammatory protein MIP-1alpha mRNA on glial cells after focal cerebral ischemia in the rat. *Neurosci. Lett.* **227,** 173–176.
107. Liu, T., Young, P. R., McDonnell, P. C., White, R. F., Barone, F. C., and Feuerstein, G. Z. (1993) Cytokine-induced neutrophil chemoattractant mRNA expressed in cerebral ischemia. *Neurosci. Lett.* **164,** 125–128.
108. Wang, X., Li, X., Schmidt, D. B., Foley, J. J., Barone, F. C., Ames, R. S., et al. (2000) Identification and molecular characterization of rat CXCR3: receptor expression and interferon-inducible protein-10 binding are increased in focal stroke. *Mol. Pharmacol.* **57,** 1190–1198.
109. Ivacko, J., Szaflarski, J., Malinak, C., Flory, C., Warren, J. S., and Silverstein, F. S. (1997) Hypoxic–ischemic injury induces monocyte chemoattractant protein-1 expression in neonatal rat brain. *J. Cereb. Blood Flow Metab.* **17,** 759–770.
110. Matsumoto, T., Yokoi, K., Mukaida, N., Harada, A., Yamashita, J., Watanabe, Y., et al. (1997) Pivotal role of interleukin-8 in the acute respiratory distress syndrome and cerebral reperfusion injury. *J. Leukocyte Biol.* **62,** 581–587.
111. Yoshimoto, T., Houkin, K., Tada, M., and Abe, H. (1997) Induction of cytokines, chemokines and adhesion molecule mRNA in a rat forebrain reperfusion model. *Acta Neuropathol. (Berl.)* **93,** 154–158.

112. Gaetani, P., Tartara, F., Pignatti, P., Tancioni, F., Rodriguez y Baena, R., and De Benedetti, F. (1998) Cisternal CSF levels of cytokines after subarachnoid hemorrhage. *Neurol. Res.* **20,** 337–342.
113. Xia, M., Qin, S., McNamara, M., Mackay, C., and Hyman, B. T. (1997) Interleukin-8 receptor B immunoreactivity in brain and neuritic plaques of Alzheimer's disease. *Am. J. Pathol.* **150,** 1267–1274.
114. Horuk, R., Martin, A. W., Wang, Z., Schweitzer, L., Gerassimides, A., Guo, H., et al. (1997) Expression of chemokine receptors by subsets of neurons in the central nervous system. *J. Immunol.* **158,** 2882–2890.
115. Xia, M. Q., Qin, S. X., Wu, L. J., Mackay, C. R., and Hyman, B. T. (1998) Immunohistochemical study of the beta-chemokine receptors CCR3 and CCR5 and their ligands in normal and Alzheimer's disease brains. *Am. J. Pathol.* **153,** 31–37.
116. Xia, M. Q., Bacskai, B. J., Knowles, R. B., Qin, S. X., and Hyman, B. T. (2000) Expression of the chemokine receptor CXCR3 on neurons and the elevated expression of its ligand IP-10 in reactive astrocytes: in vitro ERK1/2 activation and role in Alzheimer's disease. *J. Neuroimmunol.* **108,** 227–235.
117. Ishizuka, K., Kimura, T., Igata-yi, R., Katsuragi, S., Takamatsu, J., and Miyakawa, T. (1997) Identification of monocyte chemoattractant protein-1 in senile plaques and reactive microglia of Alzheimer's disease. *Psychiatry Clin. Neurosci.* **51,** 135–138.
118. Meda, L., Baron, P., Prat, E., Scarpini, E., Scarlato, G., Cassatella, M. A., et al. (1999) Proinflammatory profile of cytokine production by human monocytes and murine microglia stimulated with beta-amyloid[25–35]. *J. Neuroimmunol.* **93,** 45–52.
119. Gitter, B. D., Cox, L. M., Rydel, R. E., and May, P. C. (1995) Amyloid beta peptide potentiates cytokine secretion by interleukin-1 beta-activated human astrocytoma cells. *Proc. Natl. Acad. Sci. USA* **92,** 10,738–10,741.
120. Bruno, V., Copani, A., Besong, G., Scoto, G., and Nicoletti, F. (2000) Neuroprotective activity of chemokines against *N*-methyl-D-aspartate or beta-amyloid-induced toxicity in culture. *Eur. J. Pharmacol.* **399,** 117–121.
121. Muessel, M. J., Berman, N. E., and Klein, R. M. (2000) Early and specific expression of monocyte chemoattractant protein-1 in the thalamus induced by cortical injury. *Brain Res.* **870,** 211–221.
122. Ransohoff, R. M. (1997) Chemokines in neurological disease models: correlation between chemokine expression patterns and inflammatory pathology. *J. Leukocyte Biol.* **62,** 645–652.
123. Flugel, A., Hager, G., Horvat, A., Spitzer, C., Singer, G. M., Graeber, M. B., et al. (2001) Neuronal MCP-1 expression in response to remote nerve injury. *J. Cereb. Blood Flow Metab.* **21,** 69–76.
124. Siebert, H., Sachse, A., Kuziel, W. A., Maeda, N., and Bruck, W. (2000) The chemokine receptor CCR2 is involved in macrophage recruitment to the injured peripheral nervous system. *J. Neuroimmunol.* **110,** 177–185.
125. Fujioka, T., Kolson, D. L., and Rostami, A. M. (1999) Chemokines and peripheral nerve demyelination. *J. Neurovirol.* **5,** 27–31.
126. Kieseier, B. C., Krivacic, K., Jung, S., Pischel, H., Toyka, K. V., Ransohoff, R. M., et al. (2000) Sequential expression of chemokines in experimental autoimmune neuritis. *J. Neuroimmunol.* **110,** 121–129.
127. Halford, W. P., Gebhardt, B. M., and Carr, D. J. (1996) Persistent cytokine expression in trigeminal ganglion latently infected with herpes simplex virus type 1. *J. Immunol.* **157,** 3542–3549.