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CHEMOKINES AND VIRAL DISEASES OF THE CENTRAL NERVOUS SYSTEM

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I. INTRODUCTION

The central nervous system (CNS) has been viewed as a relatively immune privileged organ. In physiologic states, the CNS contains few, if any, leukocytes, and an absence of professional antigen-presenting cells (APC); it is barren for the expression of key immune accessory molecules such as major histocompatibility molecules (MHCs), and is fortified by an effective blood–brain barrier. Nevertheless, the past decade has witnessed a revision of this concept, as evidenced by the fact that activated T lymphocytes, regardless of their antigen specificity, migrate effectively through the blood–brain barrier (BBB), but also by the fact that the CNS contains numerous resident cells, such as microglia, cerebral endothelial cells, and astrocytes, that can, under the appropriate conditions, acquire the expression of immune accessory molecules and may function as APC (Hickey, 1999; Owens *et al.*, 1994).

Significantly, in numerous pathological conditions, immune cells are readily recruited to and accumulate in the CNS. Infection of the human

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CNS with many different viruses (e.g., human T cell leukemia virus type I [HTLV-I]; measles virus, human immunodeficiency virus [HIV] type 1; and herpes simplex virus [HSV] type 2), or of the rodent CNS (e.g., Theiler's murine encephalomyelitis virus [TMEV], mouse hepatitis virus [MHV], lymphocytic choriomeningitis virus [LCMV] and vesicular stomatitis virus) induces vigorous host inflammatory responses with recruitment of large numbers of leukocytes, particularly T lymphocytes and macrophages. This host response can be a two-edged sword that, on the one hand, is needed to control and extinguish the invading virus but, on the other, can produce immune pathology and tissue injury resulting in mental and physical debilitation, and often death, of the host organism. Therefore, increasing our knowledge of the mechanisms that control the trafficking of immune cells into the CNS, and knowledge of the subsequent interactions between these cells that contribute to CNS damage, is an important objective.

Our understanding of the factors that govern leukocyte trafficking has been advanced considerably with the discovery of a large superfamily of mostly small proteins (termed chemokines) that function as leukocyte chemoattractants in immunoinflammatory states (for reviews, see Bacon and Oppenheim, 1998; Rollins, 1997; Taub and Oppenheim, 1994). Chemokines coordinate trafficking of peripheral blood leukocytes by stimulating their chemotaxis, adhesion, extravasation, and other effector functions. In view of these properties, research efforts have turned increasingly to a focus on the possible involvement of chemokines in regulating both peripheral tissue and CNS leukocyte migration during viral infection. However, it is clear that the scope of chemokine involvement in the pathogenesis of host/viral interactions extends well beyond leukocyte migration. With studies on HIV-1 in the vanguard, new roles for chemokines and their receptors in viral infection became apparent with the demonstration that certain classes of chemokine receptors functioned as essential coreceptors for HIV-1 binding and entry into the cell. Yet more recent work has indicated that the genomes of some large viruses, belonging to the herpesvirus and poxvirus families, contain homologs of the mammalian chemokines and their receptors. These homologs may function to modulate local inflammatory responses and favor viral survival and spread. From the host perspective, accumulating evidence indicates that chemokines are plurifunctional molecules that may have a significant impact on the CNS, regulating cellular communication in the developing and the normal adult CNS (Asensio and Campbell, 1999).

Here, we review the subject of chemokines and their receptors in the evolution of viral infectious diseases of the CNS. As a prelude to the

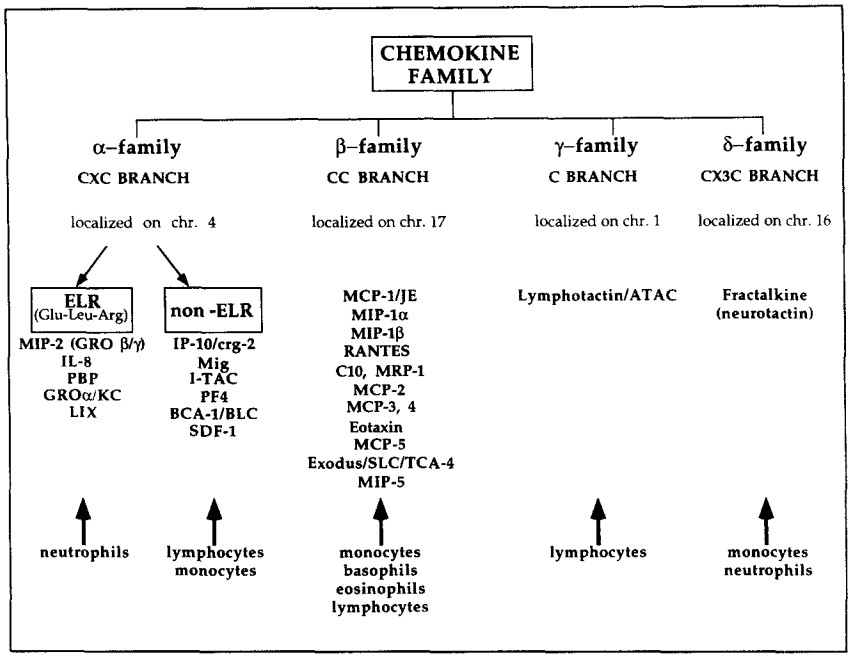


FIG 1. Classification and broad cellular specificities of the CXC, CC, C, and CX_3C chemokines. ATAC, activation-induced chemokine-related molecule exclusively expressed in $CD8^+$ T lymphocytes; BCA-1/BLC, B cell–attracting chemokine/B-lymphocyte chemoattractant; GRO, growth-regulated oncogene; IL-8, interleukin-8; IP-10, inflammatory protein 10 kD; I-TAC, interferon-inducible T cell alpha-chemoattractant; LIX, LPS-induced CXC chemokine; MCP, monocyte chemotactic protein; Mig, monokine induced by interferon-gamma; MIP, macrophage inflammatory protein; MRP-1, MIP-related protein 1; PBP, platelet basic protein; PF4, platelet factor 4; RANTES, regulated on activation-normal T cell expressed and secreted; SDF-1, stromal cell–derived factor-1; SLC, secondary lymphoid tissue chemokine; chr., chromosome.

main subject of this chapter, our discussion initially will provide the reader with a general background to the rapidly expanding area of chemokines and their receptors and the expression of these molecules in the CNS.

II. CHEMOKINES AND THEIR RECEPTORS: AN OVERVIEW

The chemokines are currently divided into 4 families (Fig. 1): the CXC or α family; the CC or β family; the C or γ family; and the CX_3C or δ family (Zlotnik *et al.*, 1999). In general, within each chemokine subfamily, the individual members show considerable homology in their

amino acid sequence and often possess overlapping chemoattractant specificity. Chemokines share a common three-dimensional structure including an NH_2-terminal loop and three antiparallel β sheets that are followed by a C-terminal α helix. The amino acid backbone of chemokines belonging to the CC, CXC, and CX_3C families contains four conserved cysteines, while the C family chemokine, lymphotactin, contains only two cysteines, which correspond to the first and third cysteines in the other groups.

Chemokines can be produced by a variety of immune cells such as T lymphocytes, macrophages, and natural killer (NK) cells, but also by organ-specific cell types such as resident glial cells in the CNS (see the discussion below). Depending on the chemokine and its cellular source, the expression of chemokines may be constitutive or inducible, and a new classification of chemokines, defined according to their expression pattern, has been proposed (Mantovani, 1999a; Sallusto *et al.,* 1999a). The regulation of chemokine expression can be mediated by soluble factors such as inflammatory cytokines, e.g., interleukin (IL)-1, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, as well as by microbial products, e.g., bacterial lipopolysaccharide (LPS) or HIV coat protein gp120 (Baggiolini, 1998; Baggiolini *et al.,* 1997; Premack and Schall, 1996; Strieter *et al.,* 1996). In contrast, counterregulatory cytokines can downregulate the production of chemokines. For example, IL-10 and transforming growth factor (TGF)-β have been shown to inhibit LPS-induced microglial cell RANTES mRNA expression and protein release (Hu *et al.,* 1999). The glucocorticoid dexamethasone is also a particularly potent inhibitor for CC and CXC chemokine production (Tobler *et al.,* 1992; Villiger *et al.,* 1992).

The biological effects of chemokines are mediated via their interaction with receptors belonging to the family of seven transmembrane (7TM)-spanning, G-protein-coupled receptors (GPCRs). All chemokine receptors contain two conserved cysteines, one in the NH_2-terminal domain and the other in the third extracellular loop; these are assumed to form disulfide bonds critical for the formation of the ligand binding pocket. In accordance with the chemokine classification, the receptors are divided into four major groups, CXCR, CCR, XCR, and CX_3CR (Table I). Individual chemokine receptors may often be promiscuous, meaning that they are capable of binding several different chemokines, and conversely, individual chemokines can often bind to several different receptors. Interaction between a chemokine and its receptor leads to generation of a coordinated series of signaling events such as mobilization and influx of Ca^{2+} and also activation of various signal transduction pathways (Premack and Schall, 1996).

TABLE I
CHEMOKINE RECEPTOR FAMILIES AND THEIR LIGANDS

Receptor family	Ligand[a]
CC family	
CCR1	MIP-1α, β, RANTES, MCP-2, MCP-3, MCP-4, MIP-5
CCR2	MCP-1, MCP-2, MCP-3, MCP-4
CCR3	RANTES, eotaxin, MCP-3, MCP-4, MIP-5
CCR4	MDC, RANTES, TARC
CCR5	RANTES, eotaxin, MIP-1α, MIP-1β, MCP-2, MCP-4
CCR6	LARC (MIP-3α)
CCR7	ELC, SLC
CCR8	I-309, TARC, MIP-1β
CCR9/10	TECK, MCP-1, MCP-2, MCP-4, MIP-1 α, eotaxin
CXC family	
CXCR1 (IL-8RA)	IL-8, GRO-α
CXCR2 (IL-8RB)	GRO-α, β, γ, IL-8, MIP-2, PBP, LIX, ENA-78
CXCR3	IP-10, Mig, I-TAC, **[MCP-4, SLC, eotaxin]**
CXCR4	SDF-1
CXCR5	BCA-1/BLC
CX_3C family	
CX_3CR1	Fractalkine-neurotactin
C family	
XCR1	Lymphotactin

[a] Chemokines in boldface type are cross-subfamily ligand-binding receptors. BCA-1/BLC, B cell-attracting chemokine/B-lymphocyte chemoattractant; ELC, Epstein-Barr virus–induced receptor ligand chemokine; ENA-78, epithelial cell–derived neutrophil-activating factor-78; GCP-2, granulocyte chemoattractant protein-2; GRO, growth-regulated oncogene; IL-8, interleukin-8; IP-10, inflammatory protein 10 kD; I-TAC, interferon-inducible T cell alpha-chemoattractant; LARC, liver- and activation-related chemokine; LIX, LPS-induced CXC chemokine; MCP, monocyte chemotactic protein; Mig, monokine induced by interferon-gamma; MIP, macrophage inflammatory protein; MRP-1, MIP-related protein 1; PBP, platelet basic protein; PF4, platelet factor 4; RANTES, regulated on activation-normal T cell expressed and secreted; SDF-1, stromal cell–derived factor-1; SLC, secondary lymphoid tissue chemokine; TECK, thymus-expressed chemokine.

A. CXC (or α-) Chemokines and Receptors

Chemokines of the CXC or α family have one amino acid (X) separating the first and second N-terminal cysteine residues. Well-known members of this family include IL-8 (interleukin-8); GRO-α,-β, and -γ (growth-regulated oncogene); MIP-2 (macrophage inflammatory protein 2); SDF-1 (stromal-derived factor-1); IP-10 (interferon-inducible

protein 10 kDa); and Mig (monokine induced by interferon-γ), (see Fig. 1). The presence or absence of an ELR motif (glutamic acid-leucine-arginine sequence) preceding the first cysteine further subdivides the CXC chemokines into ELR or non-ELR groups. Examples of some common ELR chemokines include IL-8, GRO-α, and MIP-2, while non-ELR chemokines include IP-10, Mig and SDF-1. This grouping not only reflects a structural compartmentalization, but also a functional one in that ELR CXC chemokines are predominantly chemoattractant for polymorphonuclear leukocytes and bind to the CXCR1 and CXCR2 receptors, while non-ELR chemokines are chemoattractant for T and B cells and bind to the CXCR3 or CXCR4 receptors. In addition, members of the CXC chemokine family can either promote or inhibit angiogenesis, depending on whether they are ELR or non-ELR CXC chemokines, respectively. The ELR-chemokines are generally produced during inflammatory responses provoked by bacterial infections. On the other hand, production of the non-ELR chemokines, IP-10/crg-2 and Mig, is commonly associated with viral infections where they likely function to recruit T cells and macrophages that predominate at sites of infection (Amichay *et al.,* 1996; Asensio and Campbell, 1997; Asensio *et al.,* 1999a; Carr *et al.,* 1998; Charles *et al.,* 1999; Fisher *et al.,* 1995; Lahrtz *et al.,* 1997; Nazar *et al.,* 1997; Vanguri and Farber, 1994). This scenario is borne out by the recent demonstration of pronounced antiviral activity of Mig and IP-10 when it is expressed ectopically by recombinant vaccinia viruses (Mahalingam *et al.,* 1999). The antiviral actions of Mig and IP-10 in this setting are mediated indirectly and require NK cells and IFN-α, IFN-β, and IFN-γ. SDF-1 was originally isolated from bone marrow stromal cells as a pre-B cell growth-stimulating factor. In contrast to IP-10 and Mig, SDF-1 is produced constitutively in many organs (e.g., heart, lung, and CNS) and binds to the CXCR4 receptor. Animals lacking SDF-1 or its receptor invariably die at or soon after birth and have major abnormalities affecting the immune system (an impairment in B lymphocyte development) as well as various organs including the heart and CNS (Ma *et al.,* 1998; Nagasawa *et al.,* 1996; Tachibana *et al.,* 1998; Zou *et al.,* 1998). These studies revealed that SDF-1 is a crucial mediator of cellular migration and patterning during organogenesis.

To date, 5 CXC chemokine receptors have been described and their ligands identified (see Table I) (Baggiolini *et al.,* 1997; Mackay, 1997; Moser *et al.,* 1998). Most of the early characterization of the CXC chemokine receptors was done with the IL-8 receptors CXCR1 (also known as IL-8RA) and CXCR2 (also known as IL-8RB). CXCR2 is expressed on a variety of cells including T cells, monocytes,

melanoma cells, synovial cells, neutrophils, and myeloid precursor cells. Expression of CXCR1 is restricted largely to myeloid cells and neutrophils. While CXCR2 binds all the ELR-chemokines, CXCR1 binds only IL-8. Mice lacking CXCR2 show normal development of neutrophils but have decreased numbers of myeloid progenitors (Broxmeyer *et al.,* 1996; Cacalano *et al.,* 1994). CXCR2-deficient mice do not exhibit any other overt pathologic abnormalities. CXCR3 is the receptor for several of the non-ELR CXC chemokines, IP-10, Mig and I-TAC. Surprisingly, some CC chemokines such as eotaxin, MCP-4 (Weng *et al.,* 1998), and 6Ckine (also called exodus 2 or SLC) (Soto *et al.,* 1998) also bind to CXCR3. Through this interaction, eotaxin does not activate the CXCR3 receptor but effectively blocks IP-10 binding and prevents receptor activation. Thus, these CC chemokines may serve as natural CXCR3 antagonists highlighting complexity in the regulation of chemokine actions. Human CXCR3 has been shown to be produced specifically by IL-2-activated $CD4^+$ T cells, B cells, and NK cells. $CD8^+$ T cell and NK cell clones have also been shown to express the CXCR3 receptor (Loetscher *et al.,* 1996). This suggests non-ELR chemokines such as IP-10 may exert a selective influence on Th1 and cytotoxic lymphocyte and NK cell recruitment, which may be important in the development of innate and adaptive immune responses to viral infections. CXCR4 and CXCR5 bind the other non-ELR chemokines SDF-1 and BCA, respectively. CXCR4, also known as LESTR or fusin, serves as a major coreceptor for lymphotropic strains of HIV-1 (Deng *et al.,* 1996; Feng *et al.,* 1996) and is expressed in a variety of tissues including brain, heart, liver, and colon and in nonhematopoietic cells (Lavi *et al.,* 1997; Ohtani *et al.,* 1998). CXCR4 mRNA expression is also found in murine lymphocytes, macrophages, neutrophils, and glial cells (Nagasawa *et al.,* 1998). As indicated above, disruption of the CXCR4 gene causes defects in vascular development, hematopoiesis, cardiogenesis, and neurogenesis of the cerebellum. CXCR5, originally described as BLR-1 (Burkitt's lymphoma receptor-1), is highly related to the IL-8 receptors (Dobner *et al.,* 1992). CXCR5 mRNA is expressed by B cell lymphomas and by granule and Purkinje cells of the cerebellum. Therefore, in addition to regulation of recirculating mature B lymphocytes (Forster *et al.,* 1996; Forster *et al.,* 1994; Kaiser *et al.,* 1993), this receptor may be involved in some CNS functions. However, mice lacking CXCR5 show significant phenotypic changes in lymphoid organs but not in the brain, suggesting that this receptor, unlike CXCR4, does not play an obligatory role in CNS development (Forster *et al.,* 1996).

B. CC (or β-) Chemokines and Receptors

The CC or β-chemokines contain the largest number of members and are so named because the NH_2-terminal cysteines are adjacent to each other. Individual CC chemokines may exert their actions on multiple leukocyte subtypes, including monocytes, basophils, eosinophils, T cells, dendritic cells, and natural killer cells. Well-known members of this family include MCP-1, MIP-1α, C10, eotaxin, and RANTES. A more comprehensive list of the members of this family can be found in Fig. 1. One of the most studied chemokines of this family is MCP-1, which attracts T cells and monocytes but not neutrophils (Carr *et al.,* 1994; Matsushima *et al.,* 1989). Mice with targeted disruption of the MCP-1 gene (Lu *et al.,* 1998) have defects in monocyte/macrophage recruitment in a variety of inflammatory and immunological models. Conversely, transgenic mice with expression of MCP-1 targeted to pancreatic islets (Grewal *et al.,* 1997) or oligodendrocytes in the brain (Fuentes *et al.,* 1995) show an accumulation of monocytes at these sites. Interestingly, these accumulating monocytes showed little evidence of activation, indicating that MCP-1 is an effective monocyte chemoattractant but lacks the ability to further activate these cells. Another well-studied CC chemokine is MIP-1α, which induces migration of monocytes, T lymphocytes, and eosinophils (Baggiolini *et al.,* 1994). Mice with a targeted disruption of MIP-1α show no obvious hematopoietic abnormalities; however, they are resistant to coxsackievirus-induced myocarditis and clear influenza at a delayed rate (Cook *et al.,* 1995).

To date, 9 CC receptors (CCR1-CCR9) have been identified (Table I). CCR1 was originally designated as an MIP-1α/RANTES receptor (Gao *et al.,* 1993; Gao and Murphy, 1995; Neote *et al.,* 1993) and was later shown to also bind MCP-2 and MCP-3 (Ben-Baruch *et al.,* 1995). The CCR1 receptor is mainly found on circulating mononuclear cells. Mice lacking CCR1 (receptor for MIP-1α, MIP-5, MCP-2, MCP-3, and RANTES) have a phenotype similar to that of MIP-1α deficient mice with a defect in inflammatory responses to microbial challenge such as coxsackie B and influenza viruses (Gao *et al.,* 1997). In addition, these animals have impaired trafficking of subsets of myeloid progenitor cells. The primary receptor for MCP-1, CCR2, is expressed on monocytes, myeloid precursor cells, activated T lymphocytes, and B lymphocytes but not on neutrophils (Bonecchi *et al.,* 1999; Frade *et al.,* 1997; Myers *et al.,* 1995). Mice with targeted disruption of the CCR2 receptor have defects in monocyte/macrophage recruitment in a variety of inflammatory and immunological models (Boring *et al.,* 1998; Kurihara *et al.,* 1997; Kuziel *et al.,* 1997). CCR3, which is the

eotaxin receptor, is prominently expressed by eosinophils and is involved in the recruitment of these cells during allergic reactions. CCR4 is the main receptor for RANTES and MIP-1α, while CCR5, expressed on monocytes/macrophages, T cells, and granulocyte precursors (Alkhatib *et al.,* 1996; Deng *et al.,* 1996), binds MIP-1α, MIP-1β, and RANTES. The receptors CCR6 and CCR7, originally identified as orphan receptors, bind MIP-3α, and MIP-3β and 6Ckine respectively (Varona *et al.,* 1998; Yoshida *et al.,* 1997). CCR8 binds I-309 (Goya *et al.,* 1998), TARC, and MIP-1β (Bernardini *et al.,* 1998) and is expressed in the thymus (Zingoni *et al.,* 1998). CCR9, also designated as GPR-9-6, was shown to specifically bind TECK (Zaballos *et al.,* 1999).

Compartmentalization of CC chemokine receptor expression is emerging as an important regulatory component during the adaptive immune response. CCR5, the receptor for RANTES, MIP-1β, and MIP-1α is expressed by memory and activated T cells but not by naive T cells (Bleul *et al.,* 1997). Moreover, CCR5 is preferentially expressed by human Th1 cells, in contrast to CCR4 and CCR3, which are found predominantly on Th2 cells (Bonecchi *et al.,* 1998; Qin *et al.,* 1998). Concomitant with their differential chemokine receptor expression, Th1 and Th2 cells selectively migrate in response to the corresponding chemokine ligand. More recently, Sallusto *et al.* showed that memory T cells can be distinguished with respect to their effector function in peripheral organs by their expression of the chemokine receptor CCR7 (Sallusto *et al.,* 1999b). $CCR7^+$ memory T cells retain lymph node homing receptors and lack immediate effector function, whereas $CCR7^-$ memory T cells express receptors for migration to inflamed tissues and possess effector function. CCR8, like CCR4 (Bonecchi *et al.,* 1998), is preferentially expressed by activated Th2 cells. CCR8 may be important in lymphocyte development as well as in Th2-immune responses. Polarized Th2 cells have been shown to differentially express the chemokine receptors, CCR8 (Zingoni *et al.,* 1998), CCR4 (Bonecchi *et al.,* 1998; Sallusto *et al.,* 1999a) and CCR3 (Sallusto *et al.,* 1997). In the murine system, a recent study showed that Th1 and Th2 cells expressed comparable levels of CCR1, CCR2, and CCR4 mRNA. Similar to human T helper cells, murine Th1 cells preferentially expressed CCR7 and CCR5, whereas Th2 cells expressed more CCR3 (Randolph *et al.,* 1999). In conclusion, depending on their antigenic stimulation and/or polarization toward Th1 versus Th2 subsets, chemokine receptors are differentially expressed, which, in addition to their characteristic cytokine profiles, may thus further distinguish these cells (Mantovani, 1999b).

C. C (or γ-) and CX_3C (or δ-) Chemokines and Receptors

Lymphotactin, the lone member of the C-chemokine family, is produced by activated mouse T cells (Kelner *et al.,* 1994). Lymphotactin is unique because (1) it has only two cysteines, the second and the fourth, of the four cysteines conserved in the CXC, CC, and CX_3C chemokines; and (2) the C-terminal sequence is much longer than those of other chemokines. Lymphotactin, and the subsequently reported single motif cysteine (SMC) chemokine-1 and the activation-induced, T-cell-derived, and chemokine-related molecule (ATAC), are all identical (Muller *et al.,* 1995; Yoshida *et al.,* 1995). Lymphotactin expression on activation is extremely rapid, which suggests that it has a role in the early phases of an inflammatory response. When lymphotactin is injected into peritoneum, it causes an influx of T lymphocytes and NK cells by 24h (Hedrick *et al.,* 1997). Yoshida *et al.* (1998) identified an orphan receptor, GPR5 (Heiber *et al.,* 1995), as being a high-affinity functional receptor for lymphotactin—this receptor was renamed XCR1.

The single member of the CX_3C or δ family, named fractalkine, is also unique with a novel arrangement of three amino acids separating the first two conserved cysteines. Furthermore, fractalkine exists in both soluble and membrane-bound forms (Bazan *et al.,* 1997; Pan *et al.,* 1997). This chemokine is found on activated endothelial cells and is tethered to the membrane by a mucinlike stalk that suggests a novel role for this chemokine in juxtacrine signaling. The interaction of endothelial-cell-expressed fractalkine with its receptor, CX_3CR1, on leukocytes mediates the initial capture, firm adhesion, and activation of circulating leukocytes independently of integrin or adhesion molecule involvement (Fong *et al.,* 1998). Thus, fractalkine not only induces the migration of leukocytes but also facilitates their adhesion directly to the endothelium at sites of inflammation. Fractalkine has been shown to foster the migration of monocytes, T cells, and NK cells (Hedrick *et al.,* 1997; Kelner *et al.,* 1994). The receptor for fractalkine was originally cloned from a rat brainstem cDNA library and named RBS11 (Harrison *et al.,* 1994). Subsequently, the human receptor was identified as V28 (Raport *et al.,* 1995), or CMKBLR1, which is highly expressed in the brain and in neutrophils, monocytes/macrophages, and T lymphocytes (Combadiere *et al.,* 1995). Imai *et al.* (1997) identified this molecule as being the fractalkine receptor (CX_3CR1) that is expressed mainly by NK cells and monocytes and to a lesser extent in $CD8^+$ T cells.

D. Viral Chemokine and Chemokine Receptor Homologs

Many of the large DNA viruses are infamous for their ability to undermine host immunity. Two genres of viruses, the herpesviruses and

poxviruses, were recently shown to contain open reading frames (ORFs) that encode homologs of mammalian chemokines and chemokine receptors (for detailed reviews, see Ahuja *et al.,* 1994; Dairaghi *et al.,* 1998; Lalani *et al.,* 2000; Murphy, 1994; Smith *et al.,* 1997).

Kaposi's sarcoma–associated herpes virus (KSHV) encodes three CC-like chemokines, vMIP-I, vMIP-II, and vMIP-III. The vMIP-I and vMIP-II show 60% sequence identity to each other and 43% and 52% identity to MIP-1α, while vMIP-III is more distantly related to its viral and mammalian counterparts (Stine *et al.,* 1999). Of the three viral chemokines, the function of vMIP-II is the best characterized. Receptor binding studies suggest vMIP-II is a ligand for CCR3 (Boshoff *et al.,* 1997). In addition to being a potent inhibitor of HIV infection mediated principally through this receptor (Boshoff *et al.,* 1997), vMIP-II is able to mobilize calcium and stimulate the chemotaxis of eosinophils where CCR3 is predominantly expressed (Boshoff *et al.,* 1997). In contrast to this agonist action on CCR3, vMIP-II also binds with a high degree of affinity to a number of other CC (CCR1, 2, 5) and CXC chemokine receptors (CXCR4), where it acts as an antagonist (Kledal *et al.,* 1997), as well as to CX_3CR1 (Chen *et al.,* 1998). Human monocyte chemotaxis induced by RANTES, MIP-1α, and MIP-1β is inhibited by vMIP-II, suggesting that this viral mediator may be a broad-spectrum chemokine antagonist. Recently, vMIP-I was shown to selectively bind to CCR8 (a CC chemokine receptor associated with Th2 lymphocytes), acting as an agonist and stimulating Ca^{2+} mobilization on human T cells (Dairaghi *et al.,* 1999). Interestingly, vMIP-II also binds to CCR8; however, this is associated with antagonistic actions (Sozzani *et al.,* 1998). Kaposi's sarcoma is an angioproliferative disorder, so it is interesting that in addition to deviation of the host chemokine response, vMIP-I and vMIP-II both induce angiogenesis in a chick chorioallantoic assay (Boshoff *et al.,* 1997). Thus these viral chemokines might contribute directly to the pathogenesis of the proliferative angiopathy in Kaposi's sarcoma.

In addition to these chemokine homologs, herpesviruses also contain open reading frames (ORFs) that share significant sequence conservation with the chemokine receptors. KSHV and *Herpesvirus saimiri* (HVS) contain ORF 74 in HSV, whose sequence is most similar to the human IL-8 receptors CXCR1 and CXCR2 (Ahuja and Murphy, 1993). Like CXCR1 and CXCR2, ORF 74 binds IL-8 as well as the other ELR CXC chemokines, GRO-α and PF-4 (Ahuja and Murphy, 1993). However unlike CXCR1 and CXCR2, ORF 74 can signal in a constitutive agonist–independent manner and stimulate the proliferation of kidney fibroblasts (Arvanitakis *et al.,* 1997). In addition to the stimulation of proliferation of nontransformed cells, ORF 74 signaling may also lead

to cellular transformation and tumorigenicity (Bais *et al.,* 1998). Thus, it would appear that ORF 74 may contribute to the development of the transformed cell phenotype found with KSHV-infected cells in Kaposi's sarcoma lesions.

Cytomegalovirus (CMV) is a common opportunistic pathogen of immunocompromised hosts in whom the virus exhibits tropism for leukocytes. Human and murine CMV possess genes that encode a variety of chemokine homologs (MacDonald *et al.,* 1997). The murine CMV chemokine 1 and 2 (MCK-1 and MCK-2) peptides induce calcium signaling and adherence in peritoneal macrophages (Saederup *et al.,* 1999). The human CMV gene UL146 encodes a protein designated as vCXC-1 and provides the first example of a virus-encoded CXC chemokine (Penfold *et al.,* 1999). The vCXC-1 glycoprotein shows high-affinity binding to the CXCR1 and CXCR2 chemokine receptors, inducing calcium mobilization and chemotaxis of neutrophils. Thus, the common property of the CMV chemokine homologs is to function as chemokine agonists. This in turn may promote leukocyte migration to sites of infection and may be responsible for more efficient dissemination of CMV in the host. In support of this notion, MCK-1/MCK-2 mutant CMV displays markedly reduced peak levels of monocyte-associated viremia in experimentally infected mice (Saederup *et al.,* 1999).

A gene encoding a viral homolog of CCR1 was found within the genome of the human CMV. This gene, ORF US28, of human cytomegalovirus (HCMV) was shown to bind MIP-1α,-β, MCP-1, RANTES (Gao and Murphy, 1994), and MCP-3 (Bodaghi *et al.,* 1998) with higher affinity than their cognate receptors. Thus, it is attractive to speculate that HCMV-infected cells expressing US28 may be used for sequestration of chemokines from the extracellular milieu. US28 can also serve as a coreceptor for HIV infection of CD4 T cells and may facilitate an interplay between HIV and HCMV (Pleskoff *et al.,* 1997).

The poxvirus molluscum contagiosum virus (MCV) infects humans, causing papules of the skin associated with virtually no immune response to the virus in the skin lesion. The genome of MCV contains an ORF that encodes a protein (MC148R) that is a CC chemokine homolog (Senkevich *et al.,* 1996). MC148R has homology to MIP-1α/β but does not induce chemotaxis and antagonizes MIP-1α-induced chemotaxis (Krathwohl *et al.,* 1997). Furthermore, MC148R inhibits the leukocyte response to several CC and CXC chemokines (Damon *et al.,* 1998), indicating that it can act as a broad-spectrum chemokine antagonist. Therefore, a primary function of this virus-encoded chemokine homolog might be to provide an immune evasion strategy

that acts to limit the recruitment of effector leukocytes to MCV-infected epidermal cells.

In conclusion, unquestionably these large DNA viruses encode functional homologs of chemokines and chemokine receptors. Although the precise role of the viral chemokine and chemokine receptor homologs (discussed above) in infectious processes remains to be determined, they clearly could contribute to the strategies employed by these viruses to subvert host immunity. The examples discussed here indicate that these strategies are diverse and, depending on the virus, range from wholesale suppression of leukocyte trafficking—providing immune evasion—to promoting selective leukocyte chemotaxis fostering the more efficient infection and dissemination of virus via its preferred target host cell.

III. Chemokines and their Receptors in the Central Nervous System

A concerted research effort has been mounted recently to ascertain the role of chemokines in immunoinflammatory disorders of the CNS, such as multiple sclerosis (MS). As a result, it has become clear that most cells resident in the CNS, including neurons, astrocytes, and microglia, can synthesize and secrete various classes of chemokines. Importantly, these same cells are endowed with a variety of different chemokine receptors and therefore have the potential to respond to chemokines in their local environment. As recently speculated by us (Asensio and Campbell, 1999) and by others (Mennicken *et al.,* 1999), it is likely the CNS has its own chemokine ligand/receptor network, the function of which could extend well beyond the regulation of leukocyte trafficking in antiviral and other neuroimmune responses (see Fig. 3).

A. Constitutive and Inducible Expression of Chemokines in the CNS

At present, the genes for only three chemokines, MCP-1, SDF-1, and fractalkine, are known to be expressed constitutively in the CNS. Evidence suggests MCP-1 is expressed in the cortex and hippocampus of the rat brain during development as well as in the adult animal (Pousset, 1994). More recently, MCP-1 expression at the protein level was shown in the human cerebellum, medulla oblongata, and pons (Meng *et al.,* 1999). However, the function of constitutively expressed MCP-1 in the brain is unknown.

The SDF-1 gene is found under physiological conditions in many organs including murine (Tashiro *et al.,* 1993) and human (Shirozu *et al.,* 1995) brain. SDF-1 has two isoforms, SDF-1α and SDF-1β, that are produced by alternative splicing from a single gene (Tashiro *et al.,* 1993). SDF-1α RNA is present at high levels in cultured rat astrocytes, at low levels in neurons, and is not detectable in microglia, while SDF-1β RNA is found at low levels in all these cell types (Ohtani *et al.,* 1998). Tanabe *et al.* (1997b) showed that SDF-1α was able to induce migration of microglial cells but not astrocytes, even though the SDF-1 receptor, CXCR4, was expressed on both cell types. Furthermore, human SDF-1α was able to induce calcium flux in cultured astrocytes (Bajetto *et al.,* 1999). It was hypothesized that astrocyte-secreted SDF-1 could facilitate interneural cell communication (Ohtani *et al.,* 1998). This is certainly the case during CNS development, since, as noted above, knocking out CXCR4 results in a major defect in granule cell migration and cerebellar development (Ma *et al.,* 1998; Zou *et al.,* 1998).

The CX_3C chemokine fractalkine is also found constitutively in the brain (Bazan *et al.,* 1997; Pan *et al.,* 1997). Fractalkine mRNA is expressed at high levels by neurons in the olfactory bulb, cerebral cortex hippocampus, caudate putamen, and nucleus accumbens (Harrison *et al.,* 1998; Nishiyori *et al.,* 1998; Schwaeble *et al.,* 1998). *In vitro* studies have confirmed that fractalkine is constitutively expressed by neurons and, further, can be induced in astrocytes by TNF-α and IL-1β (Maciejewski-Lenoir *et al.,* 1999). In a model of peripheral nerve injury, Harrison *et al.* have shown that fractalkine and its receptor were highly upregulated in the brain, with motor neurons being the primary source of the chemokine. Fractalkine treatment of primary cultured rat microglial cells induces vigorous increases in intracellular Ca^{2+} levels and chemotaxis, which are inhibited by an antibody against the fractalkine (CX_3CR1) receptor (Harrison *et al.,* 1998). Although the precise function of fractalkine in the CNS awaits clarification, the localization of the fractalkine receptor on microglia and the expression of its ligand by neurons clearly establish a spatial framework that could facilitate juxtacrine communication and migration between these cells (see Fig. 3) (Harrison *et al.,* 1998; Nishiyori *et al.,* 1998).

A majority of chemokines are not detectable in the CNS under physiological conditions, but are present during diverse pathologic states including, as discussed below, viral diseases. It is beyond the scope of this chapter to detail all of the findings in these different pathologic states and the reader is encouraged to consult many excellent recent reviews dealing with this subject (Asensio and Campbell, 1999; Glabinski and Ransohoff, 1999; Karpus and Ransohoff, 1998; Mennicken *et al.,* 1999; Ransohoff, 1997; Ransohoff and Tani, 1998). It is

clear from most, if not all, cases examined that glial cells represent an important source for the localized production of specific chemokines. For example, in MS, expression of IP-10 (Balashov *et al.*, 1999; Sorensen *et al.*, 1999) and MCP-1 (Simpson *et al.*, 1998; Van Der Voorn *et al.*, 1999) is prominent in astrocytes while MIP-1α expression is strongly associated with macrophage/microglia (Balashov *et al.*, 1999). In support of these clinicopathologic findings, studies *in vitro* demonstrate that expression of various CC and CXC chemokines can be induced in astrocytes and microglia by proinflammatory cytokines and LPS. Although differential expression of MCP-1 by astrocytes, and of MIP-1α by microglia, was reported for murine glial cells (Hayashi *et al.*, 1995), this was not the case with human microglia, with expression of both these chemokines being induced in these cells by LPS (McManus *et al.*, 1998). Similarly, expression of IP-10 by murine astrocytes and microglia can be induced by IFN-γ or LPS (Luo *et al.*, 1998; Majumder *et al.*, 1998; Ren *et al.*, 1998; Vanguri, 1995). In contrast to the glial cells, few inducible chemokines have been reported to be expressed by neuronal cells either *in vitro* or *in vivo*.

How does CNS chemokine expression contribute to the pathogenesis of neuroinflammatory disease states? Clearly, one key role involves recruitment of leukocytes to the brain. Differences in the chemokine gene expression patterns can be found in different neuroinflammatory diseases and correlate with a bias toward the involvement of particular leukocytes. In experimental and clinical bacterial meningoencephalitis, where CNS lesions consist predominantly of neutrophils and monocytes, there is dominant cerebral production of the neutrophil and monocyte attractant chemokines IL-8, MIP-2, and GROα, and MCP-1, MIP-1α, and MIP-1β, respectively (Lahrtz *et al.*, 1998; Spanaus *et al.*, 1997; Sprenger *et al.*, 1996). In contrast, as discussed below, in viral meningoencephalitis, mostly lymphocytes and monocytes are found in the CNS, in parallel with the cerebral expression of the chemokine genes encoding IP-10, MCP-1, and RANTES, which are effective lymphocyte and monocyte chemoattractants (Asensio and Campbell, 1997; Lahrtz *et al.*, 1997). Transgenic studies show that individual chemokines can have a specific chemoattractant "signature." Thus, CNS expression of either the GROα genes (Tani *et al.*, 1996) or the MCP-1 genes (Fuentes *et al.*, 1995) under the control of the myelin basic protein (MBP) promoter induces robust leukocyte infiltration of the CNS that is composed predominantly of either neutrophils or monocytes, respectively. Studies with intracerebral microinjection also demonstrate that the acute presence of chemokines in the CNS leads to robust and cell-specific leukocyte recruitment. For example, MIP-2 is more potent than IL-8, IP-10, or MCP-1 in stimulating polymorphonu-

clear leukocyte recruitment to the brain (Bell *et al.,* 1996), while the CC chemokine C10, when injected into the lateral ventricle, is able to induce a recruitment of macrophages, but not T cells (Asensio *et al.,* 1999b). Finally, neutralization of the chemokine MIP-1α, which is expressed in EAE, is associated with a reduction in disease severity and in CNS inflammation (Karpus *et al.,* 1995). In all, these studies indicate that the qualitative makeup of chemokine production in the CNS during disease can dictate the recruitment of selected leukocytes to the CNS, and that targeting these molecules may provide an effective therapeutic approach to suppress CNS inflammation.

B. Chemokine Receptor Expression in the Brain

The CC and CXC receptors are expressed on a number of different cell types and their expression is often regulated by exogenous and endogenous stimuli. Numerous *in vitro* and *in vivo* studies show that different neural cells express a variety of chemokine receptors under normal conditions and could therefore facilitate a direct interaction of these cells with chemokines. Neurons have prominent expression of many chemokine receptors including CXCR2, CXCR4, CCR1, CCR4, CCR5, CCR9/10, CX_3CR1, and Duffy antigen-related chemokine (DARC) (Horuk *et al.,* 1997; Klein *et al.,* 1999; Lavi *et al.,* 1998; Meucci *et al.,* 1998; Sanders *et al.,* 1998; Vallat *et al.,* 1998). Immunohistochemical staining of the human brain revealed CXCR1 is not detectable, whereas CXCR2 is expressed at high levels by subsets of projection neurons in different regions of the brain including the hippocampus, dentate nucleus, pontine nuclei, locus coeruleus, and paraventricular nucleus, and also in the spinal cord (Horuk *et al.,* 1996; Horuk *et al.,* 1997). DARC is expressed by subsets of endothelial cells and Purkinje cells in the cerebellum, suggesting that this enigmatic receptor that lacks signaling function may have multiple roles in normal and pathological physiology, perhaps as a chemokine clearance receptor (Horuk *et al.,* 1996; Horuk *et al.,* 1997). Functional CCR3, CCR5, and CXCR4 receptors, with different patterns of distribution, were demonstrated on cultured fetal human and macaque neurons (Klein *et al.,* 1999). Expression of CXCR4 has also been detected by immunohistochemical staining on subpopulations of neurons in the normal adult brain (Lavi *et al.,* 1997; Sanders *et al.,* 1998; Vallat *et al.,* 1998), while in the rat, levels of the CXCR4 receptor RNA are higher on specific neurons, which include cerebellar Purkinje cells, hippocampal hilar neurons, and cerebral cortical neurons (Wong *et al.,* 1996). A clear theme emerging from all these studies is that neurons express a

variety of chemokine receptors and that this expression in the CNS exhibits marked regional and cellular diversity, suggesting that chemokines could have differential effects on different neurons.

Other than neurons, glial cells also express a variety of chemokine receptors. The fractalkine receptor CX_3CR1 is found at high levels in rodent and human brain and shows strong localization to microglia (Combadiere *et al.,* 1998; Harrison *et al.,* 1998; Imai *et al.,* 1997). The fact that fractalkine is expressed by neurons (as noted above), and its receptor by microglia, suggests a possible important role for this chemokine and its receptor in communication between neurons and microglia. A similar scenario may follow with SDF-1, whose receptor, CXCR4, in addition to expression by neurons, is found on astrocytes (Heesen *et al.,* 1996) and on microglial cells (Tanabe *et al.,* 1997b). Finally, the CC chemokine receptors CCR1, CCR5, and CCR3 have been shown to be expressed by astrocytes and/or microglial cells both *in vitro* and *in vivo* (Boddeke *et al.,* 1999; He *et al.,* 1997; Lavi *et al.,* 1998; Tanabe *et al.,* 1997a; Vallat *et al.,* 1998; Westmoreland *et al.,* 1998; Xia *et al.,* 1998).

The expression of many of these chemokine receptors in the CNS is not static and can be dynamically regulated by various stimuli. For example, in a model of excitotoxic brain injury induced by intracerebral injection of NMDA in the rat, marked upregulation of CCR5 expression was observed by microglia and neurons (Galasso *et al.,* 1998). In brain from Alzheimer's cases, plaque-associated reactive microglia also show increased CCR5 expression (Xia *et al.,* 1998). Expression of the fractalkine receptor CX_3CR1 is increased on perineural microglia following facial nerve axotomy in the rat (Harrison *et al.,* 1998). Boddeke *et al.,* have shown, by RT-PCR (reverse transcription-polymerase chain rear) analysis, that CCR1, CCR2, and CCR5 expression in cultured rat microglial cells is significantly upregulated after LPS treatment (Boddeke *et al.,* 1999; Spleiss *et al.,* 1998). The pathophysiological significance of altered CNS chemokine receptor expression in disease states remains to be determined. However, it might be speculated that this provides yet another level of regulation in the already complex cellular communication processes mediated by the chemokines.

IV. Chemokines and their Receptors in Viral Diseases of the Central Nervous System

CNS infection, with a number of different classes of viruses, can provoke vigorous inflammatory responses with subsequent recruitment of

large numbers of leukocytes (for a detailed discussion of this topic, see Chapter 6 in this volume). In view of their functional properties, increasing interest has focused on the possible involvement of chemokines in regulating CNS leukocyte migration during viral infection. However, as discussed below, studies of HIV-1 have revealed that chemokine involvement in the pathogenesis of host–virus interactions extends well beyond leukocyte migration.

A. HIV- and SIV-Associated Neurological Disorders

The lentivirus HIV-1 and its simian counterpart, simian immunodeficiency virus (SIV), both infect the CNS and are responsible for causing neurologic disease. In the case of HIV, a vast amount of research effort focused on this virus led to the discovery of new chemokine receptors that proved to be critical for understanding the process of HIV-1 entry into cells (Bleul *et al.,* 1996; Feng *et al.,* 1996; Oberlin *et al.,* 1996). The interactions between this virus and the host CNS have also been studied extensively, and more recently the focus has turned to the chemokines and their receptors. As discussed below, the findings point to a significant role for the chemokines and the chemokine receptors in HIV-1 neuropathogenesis.

1. HIV-Associated Cognitive and Motor Disorder

Although CD4 was identified initially as a primary receptor for HIV-1 binding and entry, it was clear this could not account for many of the characteristics of HIV-1 infectivity, such as the existence of different strains of HIV-1 with selective tropism for either T lymphocytes (T-tropic) or monocytes (M-tropic). A clue to the identity of other possible cofactors for HIV-1 entry into cells came with the discovery that the chemokines MIP-1α, MIP-1β, and RANTES produced by $CD8^+$ T cells could suppress HIV-1 infection of cultured cells (Cocchi *et al.,* 1995). A seminal advance then came when it was shown that in addition to CD4, CXCR4 was required for T-tropic HIV-1 strains to infect host cells (Bleul *et al.,* 1996; Feng *et al.,* 1996; Oberlin *et al.,* 1996). The identification of CCR5 as a receptor for MIP-1α, MIP-1β, and RANTES soon led to the demonstration that CCR5 served as a prominent coreceptor for cell entry by M-tropic strains of HIV-1 (Alkhatib *et al.,* 1996; Choe *et al.,* 1996; Deng *et al.,* 1996; Doranz *et al.,* 1996; Dragic *et al.,* 1996). The factors that govern HIV-1 entry into cells are clearly very complex, and while CXCR4 and CCR5 are the major coreceptors for T-tropic and M-tropic HIV-1 strains, a large number of other chemokine receptors including CCR2b, CCR3, and CCR8, the US28 chemokine receptor homolog encoded by cytomegalovirus, as well as the orphan

chemokine receptors gpr1, gpr15/BOB, and STRL33/BONZO, can function as coreceptors for HIV-1 infection (Deng *et al.,* 1997; Farzan *et al.,* 1997; Heiber *et al.,* 1996; Liao *et al.,* 1997; Littman, 1998; Marchese *et al.,* 1994; Pleskoff *et al.,* 1997). The importance of the chemokine receptors for HIV-1 infection *in vivo* is illustrated in the case of individuals that are homozygous for a 32 base pair mutation in the CCR5 gene and who have marked resistance toward HIV infection (Biti *et al.,* 1997; O'Brien *et al.,* 1997; Theodorou *et al.,* 1997). In addition, it has been reported that individuals with mutations in the CXCR4 and CCR2 genes also exhibit a retarded progression of HIV-1-associated disease (Michael *et al.,* 1997).

A significant number of patients with HIV-1 infection suffer from a variety of neurological problems known collectively as HIV-1-associated cognitive and motor disorder, or "neuroAIDS" (see Chapter 18 in this volume). In its severest form, neuroAIDS can be responsible for subcortical dementia, memory impairment, and motor disorder. Although the neuropathology of neuroAIDS is often variable, typical features include formation of multinucleated giant cells, diffuse gliosis, myelin pallor, and increased blood–brain barrier permeability. Neurodegeneration, with disrupted synaptodendritic organization and loss of neurons, is observed in many but not all cases (Everall *et al.,* 1993). The cause of neuroAIDS and the mechanisms that lead to brain injury are not well understood, although a combination of both host- and HIV-derived toxic factors is likely to be involved (Gendelman *et al.,* 1997). It is clear, however, that productive HIV-1 infection of the CNS is largely restricted to macrophage/microglia and infrequently to neurons or astrocytes. Infection of the CNS is thought to result from the entry of HIV-1-infected lymphocytes or macrophages—the subsequent presence of HIV-1 proteins in microglia and multinucleated giant cells suggests spread of the virus from the infiltrating infected leukocytes.

As discussed above, resident cells of the CNS are well endowed with a variety of different chemokine receptors. The levels and type of coreceptor expression are likely to be pivotal determinants of HIV-1 tropism in the brain. The coreceptors for M-tropic variants of HIV-1, CCR5, CCR3, and CXCR4 are expressed by microglia (He *et al.,* 1997; Lavi *et al.,* 1997; Vallat *et al.,* 1998). Studies with primary cultures of human microglia have shown that CCR5 and CCR3 can serve as coreceptors with CD4 for HIV-1 infection, while CXCR4 is relatively ineffective in this capacity. Microglia have very low surface-expressed CD4 (Buttini *et al.,* 1998) and this may thus preclude the efficient use of CXCR4 as a coreceptor. HIV-1 infection of microglial cultures can be inhibited by the cognate ligands MIP-1β (CCR5) and eotaxin (CCR3), further verifying the key role of the microglial-expressed CC-chemokine receptors for HIV-1 entry into cells.

Interestingly, the use of both CCR3 and CCR5 for HIV-1 infection of microglia contrasts with blood-derived macrophages in which CCR5, but not CCR3, functions as a coreceptor (Alkhatib *et al.,* 1996; Deng *et al.,* 1996; Wu *et al.,* 1997). This suggests that M-tropic HIV-1 variants may arise in the brain that have distinct coreceptor requirements. In support of this, HIV-1 in the brain is typically M-tropic (Korber *et al.,* 1994; Power *et al.,* 1994). Moreover, He and colleagues (1997) demonstrated that YU2 and JRL Env proteins that are cloned directly from HIV-1-infected brain use CCR5 or CCR3, together with CD4, for virus entry into transfected cells.

A further determinant of HIV-1 entry and infection in the CNS may be the chemokines themselves. Increased levels of MIP-1α, MIP-1β, RANTES, MCP-1, IP-10, and SDF-1 have been reported in the brain or the CSF of individuals with neuroAIDS (Conant *et al.,* 1998; Kelder *et al.,* 1998; Kolb *et al.,* 1999; Letendre *et al.,* 1999; Sanders *et al.,* 1998; Schmidtmayerova *et al.,* 1996; Zheng *et al.,* 1999b). HIV-1 may directly regulate the expression of these chemokines. Thus, infection of cultured microglia and/or astrocytes markedly increases the expression of MIP-1α and MIP-1β (Schmidtmayerova *et al.,* 1996), while expression of SDF-1 RNA decreases in HIV-1-infected microglia but increases in similarly infected astrocytes (Zheng *et al.,* 1999b). In recent unpublished studies, we have observed induction of IP-10 gene expression in astrocytes in the brain of transgenic mice with astrocyte-targeted expression of HIVgp120. These transgenic mice exhibit similar neurodegenerative and other pathologic changes (e.g., astrocytosis) to neuroAIDS (Toggas *et al.,* 1994)—our findings suggest that a further action of HIVgp120 is the induction of IP-10. The CNS expression of IP-10 and other chemokines, such as MCP-1, correlates with viral load and increases with the progression of brain injury, indicating a possible role for these chemokines in the pathogenesis of neuroAIDS (Kelder *et al.,* 1998; Kolb *et al.,* 1999). Other than their potential contribution to brain injury (discussed in more detail below), the increased presence of these chemokines in the CNS during HIV-1 infection may promote, via their chemoattractant activity, the further recruitment and accumulation of T lymphocytes and macrophages in the brain. In support of this idea, HIV-Tat, protein-induced MCP-1 expression by astrocytes can stimulate the transmigration of monocytes across an *in vitro* model of the human BBB (Weiss *et al.,* 1999). Finally, since MIP-1α and RANTES can effectively inhibit CCR5 coreceptor–mediated cell entry by HIV-1 (Cocchi *et al.,* 1996; Dragic *et al.,* 1996), expression of these and other chemokines in the CNS in neuroAIDS might limit the spread of HIV-1 by antagonizing infection of target microglia.

The wide distribution of chemokine receptors in the normal and HIV-infected human brain, as well as the increased expression of their cognate ligands, not only provides an avenue for HIV entry into the brain via the microglia, but might also contribute to the neuronal injury and loss that ensue. As indicated above, current evidence supports the idea that neuronal injury and death in neuroAIDS are consequences of indirect mechanisms involving a variety of host-derived molecules—for example, cytokines and excitotoxic amino acids or HIV-encoded factors such as gp120; it is probable that the host and viral products work in combination. Loss of neurons, astrocytes, and microglia through apoptosis appears to be prominent in neuroAIDS (Adle-Biassette *et al.,* 1995; Gelbard *et al.,* 1995; Shi *et al.,* 1996) and can be induced by HIV-1 infection of these cells *in vitro* (Shi *et al.,* 1996). Analysis of a panel of diverse HIV-1 primary isolates or strains revealed that, compared with blood-derived or T-tropic HIV-1 isolates, brain-derived M-tropic HIV-1 isolates are ineffective at inducing neuronal and astrocyte apoptosis (Ohagen *et al.,* 1999; Zheng *et al.,* 1999b). Thus, the primary determinant of neurodegeneration may not be the M-tropic HIV forms constituting the major CNS reservoir of HIV-1, but rather, it resides with blood-derived viruses that are prominent during later stages of disease. Furthermore, the Env V3 region is the major determinant of neuronal apoptosis and also determines chemokine coreceptor usage for infection (Ohagen *et al.,* 1999), implicating these viral coreceptors in the effector pathways that mediate neuronal damage during neuroAIDS.

As mentioned above, the blood-derived, T-tropic HIV-1 isolates rely primarily on CXCR4 as a coreceptor, whereas the brain-derived, M-tropic isolates use CCR5 and CCR3. In view of the congruity between the induction of neuronal apoptosis and chemokine coreceptor usage, and the fact that neurons express CXCR4 (as noted above), many recent studies have focused on the possible role of CXCR4 in mediating brain injury in neuroAIDS. Hesselgesser and colleagues showed that signaling by CXCR4, following the binding of T-tropic HIVgp120, activates apoptotic cell death in human hNT neuronal cells (Hesselgesser *et al.,* 1998). This does not appear to reflect an unusual property of hNT cells, which have a transformed phenotype, as induction of apoptotic cell death is also induced in rat hippocampal neurons exposed to HIVgp120 (Meucci *et al.,* 1998). In these latter experiments, pretreatment of the neurons with the cognate ligand for CXCR4, SDF-1, partially protected against gp120-induced neuronal apoptosis, suggesting that the effects of the viral product are mediated, in part, by CXCR4 binding. More recently, purified virions from T-tropic, but not M-tropic, HIV isolates were shown to induce CD4 independent neuronal signaling and apopto-

sis that could be blocked by antibodies to CXCR4 (Zheng *et al.*, 1999a). In these studies, the purified T-tropic HIVgp120 protein proved to be far less effective in affecting neuronal signaling and cell death than the whole virions. Since the effects of the virions could be blocked by antibodies to gp120 and gp41, the findings indicate that the envelope proteins in their native state are more potent mediators than their recombinant or purified derivatives. In all, the implication from these studies, which utilized either human neuronal cell lines or highly purified rodent or human primary neuronal cultures, is that direct binding of HIV gp120 to the CXCR4 found on neurons activates apoptotic cell death in these cells. Contrary to this notion, in mixed brain-cell cultures derived from embryonic rat cerebrocortex, gp120-induced neuronal apoptosis was completely blocked by the tripeptide TKP, which blocks macrophages microglial activation (Kaul and Lipton, 1999). This blockade by TKP was specific for gp120 as SDF-1-induced neuronal apoptosis (as noted below) was unaffected by the peptide. Interestingly, gp120-induced neuronal apoptosis in this model system was also blocked by the CC chemokines RANTES and MIP-1β, which bind to CCR5, suggesting there may be regulatory cross talk between the CCR5 and CXCR4 receptors. However, the conclusion from this study is that gp120-induced neuronal apoptosis depends predominantly on an indirect pathway via activation of chemokine receptors on macrophages microglia. Many possible explanations exist as to why there are differences between these studies with respect to the observed effects of gp120's being conveyed by direct or indirect pathways. These include differences in the species and types of neuronal or brain-cell cultures used and the types, form, and levels of gp120 added to the cultures.

While the question of whether the induction of neuronal apoptosis by gp120 is consequent to interaction directly with the CXCR4 receptor on neurons or occurs indirectly, following activation of the macrophage/microglia, is unresolved, there is general agreement that HIVgp120 binding to CXCR4 can regulate a number of cellular signaling pathways (Kaul and Lipton, 1999; Meucci *et al.*, 1998; Zheng *et al.*, 1999a; Zheng *et al.*, 1999b). These include inhibition of cyclic AMP; activation of phosphoinositol (PI) hydrolysis, which is partially prevented by antibody to CXCR4 (Zheng *et al.*, 1999a; Zheng *et al.*, 1999b); activation of p38 mitogen–activated kinase (p38 MAPK) (Kaul and Lipton, 1999); and increased Ca^{2+} mobilization (Meucci *et al.*, 1998). HIV gp120 neuronal apoptosis can be inhibited by inhibitors of p38 MAPK (Kaul and Lipton, 1999) and by calcium/calmodulin-dependent protein kinase II, protein kinase A, and protein kinase C (Zheng

et al., 1999a), linking neuronal apoptosis, in part, to these signal transduction pathways.

The preceding discussion indicates that HIV gp120 neurotoxicity likely involves, in part, the chemokine receptor CXCR4, raising the possibility that HIV-1 subverts a key physiological cell death pathway. As we noted above, SDF-1/CXCR4 signaling is crucial for normal cerebellar neuronal migration and patterning during CNS development. SDF-1 is expressed constitutively in the adult CNS predominantly by astrocytes and to a lesser degree by neurons (Ohtani *et al.,* 1998), raising the likelihood that this chemokine may regulate physiological processes in the adult brain. In support of this, SDF-1α regulates a number of signal transduction pathways in neurons, causing decreased cyclic AMP, increased PI hydrolysis, activation of p38MAPK, and increased Ca^{2+} mobilization (Kaul and Lipton, 1999; Meucci *et al.,* 1998; Zheng *et al.,* 1999a). Functionally, exposure of hippocampal neurons to SDF-1 results in increased synaptic transmission, which is blocked by antibodies to CXCR4 (Zheng *et al.,* 1999a). The similar effects of SDF-1 and HIV gp120 in regulating CXCR4 signaling raise the question as to whether SDF-1 might also induce neuronal apoptosis. Here, the findings have been conflicting. Four studies revealed that SDF-1 promoted increased neuronal apoptosis (Hesselgesser *et al.,* 1998; Kaul and Lipton, 1999; Zheng *et al.,* 1999b) and activation of a key cell death gene, caspase 3 (Zheng *et al.,* 1999a). In contrast to these findings, Meucci and colleagues reported that, following treatment with SDF-1, there was marked protection of neurons from culture- and HIV gp120-induced apoptosis (Meucci *et al.,* 1998). The reason for these discrepancies is not known but again likely reflect differences in cell preparations and culture conditions.

2. SIV Encephalitis

Similar to HIV, SIV infection of macaques can lead to the development of an AIDS-associated encephalitis that is virtually indistinguishable from neuroAIDS (Raghavan *et al.,* 1999; Sasseville and Lackner, 1997). Both T-tropic and M-tropic SIV variants have been isolated and, like neuroAIDS, macrophage/microglia constitute the primary infected target cell population in the brain of SIV-infected monkeys. The host immune system plays a major role in the infection of the CNS with SIV, where perivascular macrophages are continuously replaced via recruitment from the circulating monocyte pool through the blood–brain barrier (Lane *et al.,* 1996). Thus, infected T cells and monocytes likely enter the CNS parenchyma during the asymptomatic phase of infection.

In addition to leukocyte and endothelial adhesion molecules, chemokines could be critical components involved in SIV infection of the brain. Using immunohistochemical staining to investigate chemokine expression in the encephalitic brain of SIV-infected macaques, Sasseville and coworkers reported upregulation in the expression of a number of chemokines, including MIP-1α, MIP-1β, RANTES, MCP-3, and IP-10, by vascular endothelium and/or perivascular mononuclear cells (Sasseville *et al.,* 1996). In contrast to neuro AIDS, MCP-1 was not elevated in the SIV-encephalitic monkey brain. With the exception of MCP-1, expression of these proinflammatory chemokines in the SIV-infected brain is similar to that found in the human brain in neuroAIDS (as noted above). However, like neuroAIDS, the *in vivo* function of these chemokines in SIV-encephalitis remains unknown; it is reasonable speculation however, to suggest that leukocyte recruitment to the SIV-infected CNS may be directed by these chemokines. In a follow-up study by this group, examining chemokine receptor expression in SIV-AIDS encephalitis, the chemokine receptors CCR3, CCR5, CXCR3, and CXCR4 were found to be expressed by inflammatory cells within perivascular lesions (Westmoreland *et al.,* 1998). In addition, expression of CCR3, CCR5, and CXCR4 was detected on subpopulations of large hippocampal and neocortical pyramidal neurons and on glial cells in both the normal and the SIV-encephalitic brain. Again, these findings show an overlap with those reported for the normal and the HIV-infected brain in humans (as noted above). M-tropic strains of SIV utilize CCR5 as a coreceptor with CD4 for infection of macrophages (Chen *et al.,* 1997; Deng *et al.,* 1997). Expression of CCR5 is enriched on microglial cells in the macaque brain and may therefore provide the molecular framework for infection by SIV in the monkey CNS.

Overall, the preceding discussion highlights the overlapping expression patterns and potentially similar roles for the chemokines and their receptors in the pathogenesis of SIV-encephalitis and neuroAIDS. This idea is further underscored by a recent study in which chemokine receptor expression and signaling were examined in cultured neurons derived from either macaque or human brains (Klein *et al.,* 1999). Thus, both the qualitative pattern of expression and the functional behavior of the chemokine receptors, CCR3, CCR5, and CXCR4, in the two species are remarkably alike.

B. Virus-Induced CNS Demyelinating Diseases

In humans, multiple sclerosis is the most common and the best-known CNS demyelinating disorder. MS is thought to be an autoim-

mune T-cell-mediated disease that is targeted at the myelin sheath. The etiology of MS is unknown (Hafler, 1999), although at one time or another, different viruses have been implicated in its pathogenesis, including Epstein-Barr virus (EBV), measles virus, and recently, HHV-6. The human T lymphotropic virus type I (HTLV-1) infection of the CNS can cause a progressive inflammatory demyelinating disease and shares many similarities with MS. In view of a possible viral role in MS, research has focused on several experimental viral models associated with the development of neuroimmune responses and primary demyelination that show many clinicopathologic similarities with MS. Among the best-known and most widely studied of these experimental models are Theiler's murine encephalomyelitis virus (TMEV)- and murine hepatitis virus (MHV)-induced demyelinating disease.

1. HTLV-1-Associated Myelopathy / Tropical Spastic Paraperesis (HAM / TSP)

HTLV-1 is the etiologic agent for adult T cell leukemia (ATL) and for HAM/TSP, which shows similar features to primary progressive MS (Calabresi *et al.,* 1999). HAM-TSP is a chronic progressive neurological disorder characterized by a perivascular demyelination and axonal degeneration, along with the presence of inflammatory infiltrating cells such as macrophages and $CD8^+$ T cells in the damaged areas (Moore *et al.,* 1989; Umehara *et al.,* 1993; Wu *et al.,* 1993). These infiltrating cells produce several inflammatory mediators including the chemokine MCP-1 (Umehara *et al.,* 1993). In addition, HTLV-1-infected T cell lines produce a number of chemokines including SDF-1, RANTES, MIP-1α, and MIP-1β (Arai *et al.,* 1998; Baba *et al.,* 1996; Mendez *et al.,* 1997). HAM/TSP patients have a high frequency of $CD8^+$ CTL in their peripheral blood and cerebrospinal fluid (CSF). Biddison and coworkers have shown that HTLV-I-specific $CD8^+$ CTL clones secrete MIP-1α, MIP-1β, and IL-16 (Biddison *et al.,* 1997). Thus, these HTLV-1 CTLs may be an important source of chemokines in HAM/TSP, where they might contribute to the pathogenesis of this disorder. Consistent with this, elevated MIP-1α protein was found in the CSF in two of three patients with HAM/TSP (Miyagishi *et al.,* 1995). A further clinical study showed that MCP-1 was detectable on perivascular inflammatory cells and the vascular endothelium in active–chronic lesions of spinal cords of HAM/TSP patients (Umehara *et al.,* 1996).

2. MHV Encephalomyelitis

MHV belongs to the coronaviruses, a ubiquitous group of positive-stranded RNA viral pathogens of man and animals associated with a variety of respiratory, gastrointestinal, and neurological disorders.

CNS infection of susceptible murine hosts with neuro-adapted strains of MHV leads to a robust initial recruitment of leukocytes to the brain that follows an early peak of viral replication. This acute encephalomyelitis phase, with its resultant tissue injury, often leads to death of the host. However, in surviving animals, further viral replication is controlled by the host response and a chronic mononuclear cell inflammation, which occurs in the brain and spinal cord, is linked to a progressive demyelinating disease (Buchmeier and Lane, 1999; Lane and Buchmeier, 1997; Weiner, 1973). The mechanisms of demyelination in this model are not clear, although there is a requirement for a competent immune response.

In a study of the kinetics and histological localization of chemokine gene expression in the brain and spinal cord of MHV-infected mice, Lane and colleagues showed that a number of α- and β-chemokines were expressed during acute or chronic stages of MHV-infection (Lane *et al.,* 1998). Expression of the transcripts for IP-10, MIP-2, MCP-1, MCP-3, MIP-1β, and RANTES overlapped with the occurrence of acute viral encephalomyelitis, being present by day 3 postinfection and peaking at day 7 postinfection. During the chronic demyelinating phase of the disease, both IP-10 and RANTES transcripts remained elevated. IP-10 RNA colocalized with viral RNA and was present in astrocytes and microglia associated with demyelinating lesions (Lane *et al.,* 1998). With the exception of RANTES, a similar pattern of chemokine expression was observed with cultured astrocytes infected with active, but not UV-inactive, MHV, indicating that viral replication in these cells can directly stimulate chemokine gene expression. Therefore, in MHV infection of the brain, early viral-induced chemokine gene expression by resident CNS cells such as astrocytes might promote the recruitment of T lymphocytes and macrophages and contribute to the maintenance of the chronic inflammatory response leading to demyelination.

Further studies in the MHV model using CD4 KO and CD8 KO mice show an important role for $CD4^+$ T-cells in the pathogenesis of inflammatory disease and demyelination (Lane *et al.,* 2000). Thus, MHV-infected CD4 KO mice have a marked reduction in the number of activated macrophage/microglia within their brains and spinal cords and significantly less demyelination. Concomitant with the reduction in CNS inflammatory disease in the MHV-infected CD4 KO mice, lower levels of RANTES, but not other chemokine transcripts and protein were found indicating that $CD4^+$ T-cells represent one major source of RANTES in the CNS during MHV encephalomyelitis. Administration of RANTES neutralizing antisera to MHV-infected mice is associated with a significant reduction in macrophage infiltration and

demyelination compared to control mice (Lane *et al.,* 2000). These data clearly indicate that $CD4^+$ T-cells have a pivotal role in accelerating CNS inflammation and demyelination within infected mice possibly by regulating RANTES expression.

3. Theiler's Murine Encephalomyelitis Virus-Induced Demyelinating Disease

TMEV-induced demyelinating disease is characterized by CNS mononuclear cell infiltration resulting in a chronic $CD4^+$ T-cell-mediated demyelinating disease (for more discussion, see Chapter 7 in this volume). A study of the expression of CC and CXC chemokines in the CNS throughout the disease course showed that expression of C10, IP-10, MCP-1, MIP-1α, MIP-1β, and RANTES mRNA transcripts overlapped with the development of disease (Hoffman *et al.,* 1999). Expression of MIP-1α and MIP-1β protein was also detectable, being present in the spinal cord before the onset of disease and persisting throughout disease progression. These findings are somewhat reminiscent of those reported for MHV encephalomyelitis (discussed above) and reiterate the theme that chemokine expression in these viral-induced immune-mediated demyelinating diseases is a complex process that is clearly associated with disease progression. Identifying the key chemokine protagonists in TMEV-induced demyelinating disease awaits clarification.

C. Viral Meningoencephalitides

Meningoencephalitis represents one of the most devastating consequences of viral infection of the CNS, in which a concerted immunoinflammatory response by the host produces severe injury to the brain, leading to debilitating neurological disease and often death. In the viral meningoencephalitides, the specific mechanisms underlying the localization, extravasation, and activation of immune cells in the CNS and the subsequent interactions between these cells are not well understood.

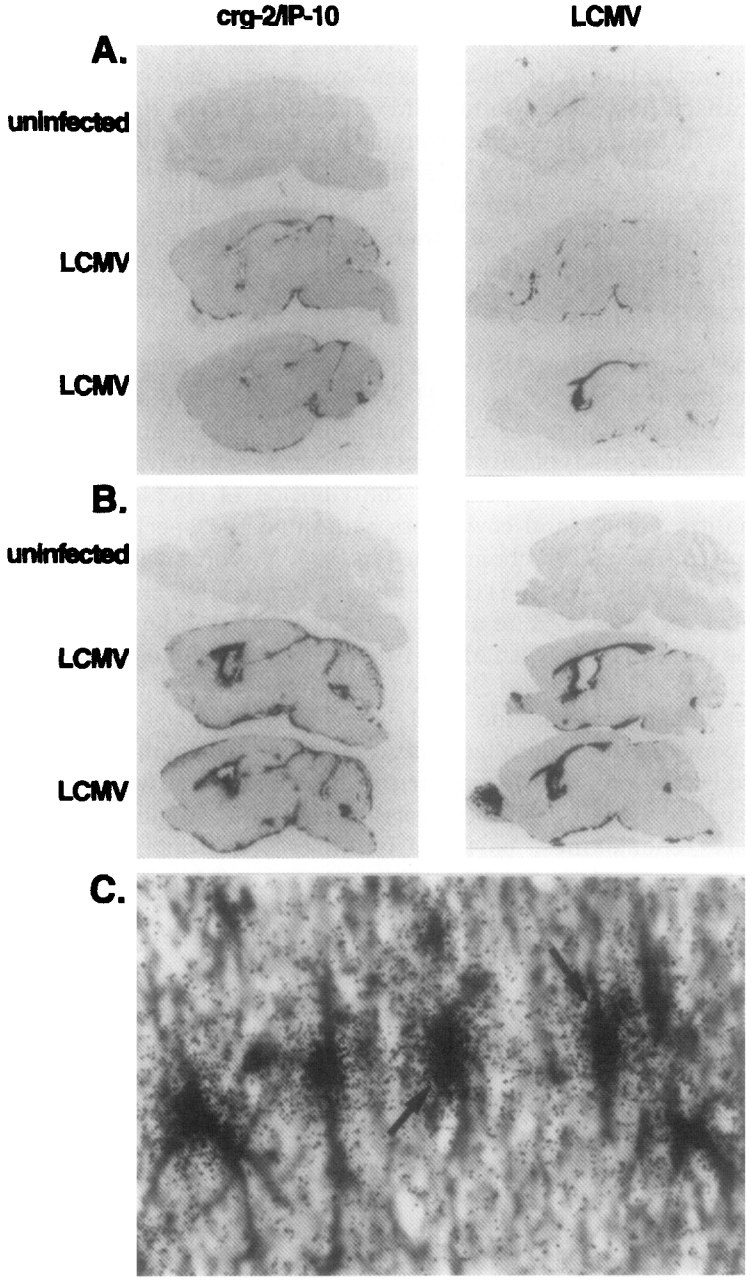
1. Lymphocytic Choriomeningitis

Studies from our own laboratory examined chemokine gene expression in lymphocytic choriomeningitis (LCM) that was induced by intracranial inoculation of mice with LCMV (Asensio and Campbell, 1997; Asensio *et al.,* 1999a). Following inoculation, LCMV infects and rapidly replicates in the meninges, the choroid plexus, and the ependymal membranes lining the ventricles. In this very well characterized model, immunocompetent adult mice infected with LCMV develop an

acute monophasic disease characterized by the presence of infiltrating mononuclear cells in these same regions of the brain, and this leads to convulsive seizures and death by 6–8 days later (Buchmeier *et al.,* 1980; Doherty *et al.,* 1990). Infiltrating cells are predominantly T lymphocytes as well as macrophages. MHC class-I-restricted anti-LCMV $CD8^+$ cytotoxic lymphocytes (CTLs), in addition to clearance of the virus, are also the primary effectors of LCM (Buchmeier *et al.,* 1980; Doherty *et al.,* 1990). Immune-compromised mice that are unable, or fail, to mount anti-LCMV $CD8^+$ CTL responses do not develop immune pathology in the brain and survive.

We hypothesized that chemokines may be important regulatory signals for the cerebral recruitment and extravasation of leukocytes in LCM (Asensio and Campbell, 1997; Asensio *et al.,* 1999a). In examining this, we observed that the pattern of chemokine gene expression in LCM is dynamic and complex, with often overlapping expression of a number of different subclasses of chemokine genes. Thus, by day 3 postinfection the expression of a number of chemokine genes is evident, including C10, MCP-3, MIP-1β, MCP-1, CRG-2/IP-10, and RANTES. By day 6 postinfection the expression of all these chemokine genes increases markedly and the expression of the lymphotactin gene is also evident in the brain. A qualitatively similar but markedly decreased level of chemokine gene expression is observed in the brain of LCMV-infected athymic mice. A similar pattern of cerebral chemokine gene expression is also found in LCMV-infected IFN-γ KO mice, although the levels of expression in the absence of IFN-γ are about 50% of those in LCMV-infected wild-type controls. In both euthymic and athymic mice, expression of IP-10 was predominant at both early and late time points after infection and preceded detectable increases in proinflammatory and interferon cytokine gene expression and CNS leukocyte recruitment in euthymic mice. In all,

FIG 2. The CXC chemokine IP-10 is expressed at high levels in the brain, following infection by many viruses. In this example mice were infected intracranially with LCMV, and the viral nucleoprotein (NP) and IP-10 RNA expression in the brain were analyzed by *in situ* hybridization. High expression of IP-10 RNA was apparent by day 3 (A) postinfection and increased further by day 6 (B) postinfection. Regional expression of IP-10 RNA, on the whole, overlapped with sites of viral infection, as indicated by the expression of the LCMV-NP gene. However, parenchymal expression of the IP-10 gene was also found in the absence of local LCMV-NP expression. By using dual-label analysis to identify the cellular localization of IP-10 RNA expression, these parenchymal cells were identified as GFAP-positive astrocytes (C; arrows). From Asensio, V. C., Kincaid, C., and Campbell, I. L. Chemokines and the inflammatory response to viral infection in the central nervous system with a focus on lymphocytic choriomeningitis virus. *J. Neurovirol.* 5:65–75, 1999. Copyright 1999, American Society for Investigative Pathology; with permission.


crg-2/IP-10
LCMV
A.
uninfected
LCMV
LCMV
B.
uninfected
LCMV
LCMV
C.

these observations, together with the finding that CRG-2/IP-10, a prominently expressed chemokine gene in many different CNS viral infections, is expressed by resident CNS cells including astrocytes (see Fig. 2), suggest that activation of chemokine gene expression may be a direct, early, and localized host response to LCMV infection. These findings are consistent with the proposed involvement of chemokines as key signaling molecules for the subsequent migration of leukocytes to the CNS, following LCMV infection. However, formal demonstration of the precise chemotactic, and possibly of other functions of chemokines in LCM, presently awaits verification.

2. HSV Encephalitis

Herpes simplex virus (HSV) is a common etiologic agent of acute focal encephalitis. Pathologically, in HSV encephalitis, leukocytes infiltrate the subarachnoid space as a hallmark of meningitis and encephalitis, while CSF analysis typically reveals a predominantly lymphocytic pleocytosis. To evaluate the possible contribution of chemokines to HSV encephalitis, Rosler and coworkers examined the spectrum, quantity, and time course of CSF chemokines in three patients with proven HSV type 1 encephalitis (HSE-1) (Rosler *et al.,* 1998). High chemokine levels were present in the CSF of all HSE-1 patients. Peak levels occurred at the time of admission, with MCP-1 being greater than either MIP-1α or RANTES. IL-8 levels were elevated at 4 to 8 hours after admission. By contrast, plasma chemokine levels were considerably lower than CSF levels, pointing to the localized nature of the CNS chemokine response in these HSE-1 patients. A comparison of MCP-1 levels with clinical status revealed a high reciprocal correlation. This single clinical study implicates chemokines, particularly MCP-1, in the pathogenesis of HSE-1 and suggests these mediators may be useful indicators to determine the stage and severity of HSE-1.

Experimental studies of HSV-1 in mice, following ocular infection, highlight the rapid and sustained upregulation of a number of chemokine genes including GRO-α, MIP-1β, MIP-2, MCP-1, IP-10, and RANTES (Carr *et al.,* 1998; Thomas *et al.,* 1998). HSV stromal keratitis, associated with productive infection in the eye, results in significant accumulation of PMN, which may be driven by the presence in particular of the CXC chemokines GRO-α and MIP-2 (Thomas *et al.,* 1998).

3. Paramyxovirus and Enterovirus Meningitis

A clinical survey of chemokine expression in CSF in patients with viral meningitis, due to infection with paramyxoviruses or enteroviruses, implicated IP-10 and MCP-1 as important contributory factors in the accumulation of activated T cells and monocytes in the

CNS (Lahrtz *et al.,* 1997). Importantly, this study formally demonstrated that the IP-10 and MCP-1 levels in CSF correlated with leukocyte chemotactic activity. MCP-1 was identified as an important chemoattractant for PBMCs while the combination of MCP-1 and IP-10 was required for migration of activated T cells. This study clearly establishes a direct link between the CNS expression of the chemokines IP-10 and MCP-1 and CNS leukocytosis in viral meningitis.

In experimental studies, the paramyxovirus Newcastle disease virus (NDV) induces IP-10 gene expression in infected astrocytes and microglia detectable by 3 hours postinfection (Vanguri and Farber, 1994). UV-inactivated NDV proved to be as effective as live virus in inducing IP-10 gene expression and was not blocked by the protein synthesis inhibitor cycloheximide, indicating that the IP-10 gene functions as an immediate early response gene, following NDV infection. Similar to NDV, measles virus (MV) is a paramyxovirus that induces IP-10 gene expression in human glioblastoma cells (Nazar *et al.,* 1997). The promoter requirements for the induction of IP-10 gene expression are similar for IFN-γ and MV; however, these two stimuli use different combinations of DNA binding factors, with STAT 1 or NFκB being more important in the direct induction of IP-10 by IFN-γ or MV, respectively. While it is presently unknown whether these paramyxoviruses induce the expression of other chemokine genes, their potent ability to directly induce IP-10 expression by glial cells may constitute a significant CNS source of production of this chemokine during viral meningitis.

D. Other Viral Diseases

Mouse adenovirus-type 1 (MAV-1) is a double-stranded DNA virus that causes a fatal hemorrhagic encephalopathy in C57B1/6 mice within 4–6 days (Guida *et al.,* 1995), along with infection of cerebrovascular endothelial cells. Chemokine gene expression was investigated and prominent induction of IP-10 was observed in the spleen and CNS of susceptible mice, whereas MCP-1 and MIP-2, respectively, were found in the spleen and brain of resistant mice (Charles *et al.,* 1999). Vascular endothelium and CNS glia were identified as sites of IP-10 mRNA expression in susceptible animals.

V. Concluding Remarks

In the normal mammalian CNS, the number of leukocytes present in the brain is scant. However, these cells are attracted to, and accu-

mulate in, a variety of pathologic states, many involving viral infection. Although leukocyte migration into local tissue compartments such as the CNS is a multifactorial process, it has become clear that chemokines are pivotal components of this process, providing a necessary chemotactic signal for leukocyte recruitment. Generation of this signal in CNS viral infection may involve localized production of proinflammatory chemokines by cells intrinsic to the brain, including the astrocytes and microglia. The activated glia have the potential to produce a spectrum of CC and CXC chemokines that may vary, depending on the nature of the invading pathogen. This in turn will determine the qualitative makeup of the leukocytes recruited to the brain. It is important to note, however, that how a chemokine that is secreted by a parenchymally located glial cell is "sensed" by circulating peripheral leukocytes is unclear at this time.

Roles beyond leukocyte chemoattraction are implied for the chemokines and their receptors. Studies of HIV highlight the dynamic relationship between chemokine receptor usage and tropism for different isolates of HIV. On the other hand, the fact that chemokine receptor ligands are effective inhibitors of HIV entry into target cells suggests their local production may serve as a host response to limit further spread of the infectious agent. Some chemokines such as IP-10 are documented as having direct antiviral functions. Whether this is important in CNS defense against viral infection where IP-10 is highly expressed, and whether any other chemokines have an antiviral function, remain open questions.

A further consequence of the localized production of chemokines in the brain is likely the direct modulation of CNS cell function. Various classes of chemokine receptors are expressed by CNS cells including neurons and the glia. In many respects the CNS possesses its own chemokine network permitting autocrine and paracrine regulation of cellular activity. Chemokines are implicated in many physiological functions including development, cell growth, angiogenesis, and cellular migration. Perturbation of the CNS chemokine network, resulting from viral infection, could therefore either be beneficial by promoting reparative processes or detrimental by contributing to tissue injury and loss.

In conclusion, it is now apparent that, like their proinflammatory cytokine counterparts, the chemokines are important plurifunctional mediators in the host response to viral infection of the CNS (see Fig. 3 and color section). As such, a greater understanding of the neurocellular and viral targets and functions of the chemokines holds the promise of revealing new molecular focal points for therapeutic inter-

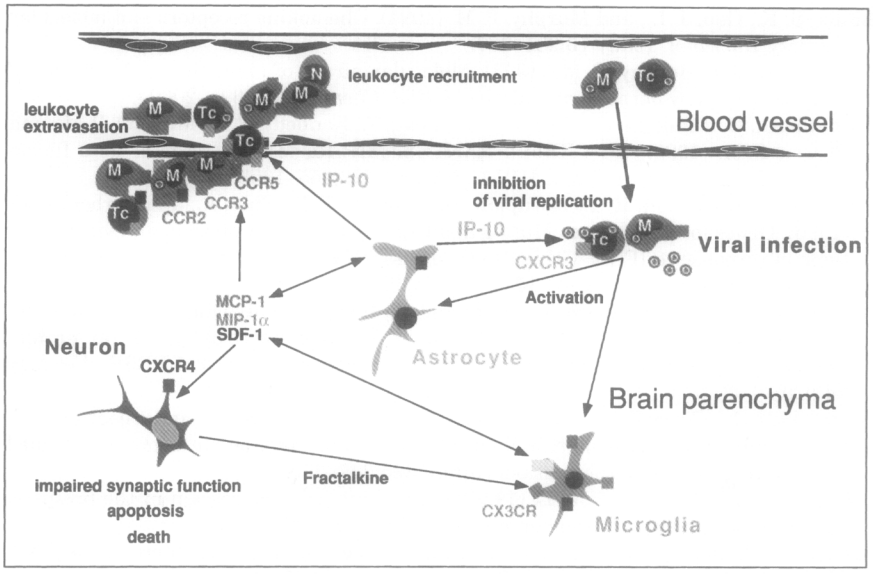


FIG 3. [For color reproduction, see color section.] Chemokines are potentially multifunctional effectors in CNS viral infections. In this schema, initial infection of the CNS with a virus results in the localized production of chemokines by reactive astrocytes and microglia. Some chemokines such as SDF-1 and fractalkine are produced constitutively in the CNS and their production may also be altered by viral infection. Increased chemokine production may then promote the further recruitment and extravasation of antiviral immune effector cells from the periphery. Chemokine receptors are widely expressed by neuronal and glial cells and additional functions of the chemokines are likely. These may include direct antiviral actions (for example, by IP-10), and intercellular communication (for example, neuronally derived fractalkine can stimulate microglia). In addition, chemokines may exert detrimental actions; for example, SDF-1 can impair neuronal activity and stimulate apoptosis of these cells. M, macrophage; Tc, T lymphocyte; N, neutrophil.

vention that could more effectively control CNS viral infections and prevent tissue injury.

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