American Journal of Pathology, Vol. 149, No. 5, November 1996

Short Communication

Chemokine Expression in Simian Immunodeficiency Virus-Induced AIDS Encephalitis

Vito G. Sasseville,* Martha M. Smith,* Charles R. Mackay,† Douglas R. Pauley,* Keith G. Mansfield,* Douglas J. Ringler,† and Andrew A. Lackner*

From the Division of Comparative Pathology, New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts, and LeukoSite, Inc.,† Cambridge, Massachusetts*

The pathogenesis of neurological dysfunction associated with human immunodeficiency (HIV)-1 infection is uncertain. However, the presence of macrophage infiltrates in the central nervous system is a key feature of HIV encephalitis and is correlated with HIV-associated dementia. Moreover, it has been demonstrated that HIV-infected monocyte/macrophages can produce toxic substances that may play a critical role in the development of HIV-associated dementia. However, the exact mechanisms responsible for HIV infection and leukocyte recruitment to the central nervous system remain speculative. Similar to HIV-infected patients, simian immunodeficiency virus (SIV)-infected macaque monkeys develop immunosuppression and acquired immune deficiency syndrome (AIDS)-related inflammatory disorders, including AIDS encephalitis. In this study, we demonstrate that encephalitic brain from SIV-infected animals has elevated immunohistochemical expression of the C-C chemokines, macrophage inflammatory protein-1α and -β, RANTES, and monocyte chemotactic protein-3, and the C-X-C chemokine interferon-inducible protein-10. These findings suggest that one or all of of these chemokines could be involved in leukocyte recruitment to the brain in SIV-infected macaque monkeys. (Am J Pathol 1996, 149:1459–1467)

Human immunodeficiency virus (HIV)-associated dementia is a clinical disorder characterized by cognitive, motor, and behavioral changes[1]. A correlation between HIV-associated dementia and the presence of HIV in the central nervous system (CNS),[2] dendritic pathology,[3] and neuronal loss[4] has been suggested. However, the pathogenesis of HIV-associated dementia remains a mystery. Recently, a stronger association between HIV-associated dementia and differences in the spatial pattern of neurons[5] and increased numbers of macrophages in the brain has been demonstrated.[6]

Similar to HIV-infected patients, many simian immunodeficiency virus (SIV)-infected macaque monkeys develop primary lentivirus-induced encephalitis.[7–9] HIV and SIV encephalitis is characterized by parenchymal and perivascular infiltrates of macrophages/microglia and multinucleate giant cells most commonly found in the white matter tracts of the cerebrum and brain stem and the deep gray matter of the CNS.[8–11] Macrophages/microglia in the CNS are the principal target for HIV and SIV, and abundant viral nucleic acid and antigen are observed in these cellular infiltrates.[10,12] Although infection of endothelium, astrocytes, oligodendrocytes, and neurons has been demonstrated *in vitro*, infection of these cells *in vivo* is either restricted or nonexistent.[13–15] Thus, neuronal dysfunction observed in many patients with HIV-associated dementia is most likely a result of factors associated with macrophage/microglia infection and subsequent development of

Supported by Public Health Service Awards AI25644, NS33820, NS30769, and RR00168.

Accepted for publication July 15, 1996.

Address reprint requests to Dr. Vito G. Sasseville, Harvard Medical School, New England Regional Primate Research Center, PO Box 9102, Southborough, MA 01772-9102.

macrophage infiltrates in the CNS.[6] However, pathogenetic mechanisms responsible for the recruitment of monocytes to the CNS in HIV-infected patients remain speculative.

It is well established that the sequential interactions of the selectins, integrins, and members of the immunoglobulin gene superfamily and their corresponding ligands are crucial for leukocyte rolling, firm adhesion, and transendothelial migration at tissue injury sites.[16] In addition to the interactions of leukocyte and endothelial adhesion molecules, monocytes are also activated and migrate in response to chemotactic gradients elicited from inflammatory sites.[16,17] Pivotal components of this process are a group of chemotactic cytokines, termed chemokines. Chemokines are structurally related, low-molecular-weight, proinflammatory proteins induced by numerous inflammatory and resident cells.[17] They are divided into two subfamilies based on the presence or absence of an amino acid separating the first pair of cysteines (α or C-X-C and β or C-C chemokines). Generally, the C-X-C chemokines stimulate and are chemoattractant for neutrophils, whereas the C-C chemokines activate and attract monocytes, lymphocytes, and eosinophils.[17–20] Moreover, many of the chemokines have been shown to attract specific subsets of mononuclear cells.[18,21]

In this study, we demonstrate that encephalitic brain from SIV-infected animals has elevated immunohistochemical expression of the C-C chemokines, macrophage inflammatory protein (MIP)-1α and -β, RANTES (regulated upon activation normal T cell expressed presumed secreted) and monocyte chemotactic protein (MCP)-3, and the C-X-C chemokine interferon-inducible protein (IP)-10. These findings suggest a role for one or multiple chemokines in the pathogenesis of acquired immune deficiency syndrome (AIDS) encephalitis.

Materials and Methods

Animals and Virus

Brain tissue from twenty-one rhesus monkeys (*Macaca mulatta*), five cynomolgus monkeys (*M. fascicularis*), two pigtailed macaques (*Macaca nemestrina*), and one Barbary macaque (*Macaca sylvana*) was collected at death and used for immunohistochemical analysis. The survival time, age, and related animal data are in Table 1. Eighteen of these animals were experimentally infected with SIV. Ten of the twelve animals with SIV encephalitis were inoculated intravenously with either uncloned SIVmac251, molecularly cloned SIVmac239, or an uncloned macrophage tropic variant of SIVmac239, termed SIVmac239/316.[22] The remaining two animals were inoculated intracerebrally with SIVsmm strain B670 (kindly provided by Dr. Michael Murphey-Corb, Tulane University). The procedures associated with the experimental SIV infection of many of these macaques have been described in detail previously.[22–24] Although different manifestations of disease have been observed with these isolates of SIV, all of them have been associated with SIV encephalitis terminally.[8,10,22,24]

Several control groups were also examined. The first group consisted of six SIV-infected animals without encephalitis and the second contained six uninfected rhesus monkeys. The last group consisted of brain tissue from five cynomolgus monkeys with experimental allergic encephalomyelitis (EAE) evaluated as a positive control of non-SIV-induced neurological disease (kindly provided by Drs. Claude Genain and Stephen Hauser). The protocol for in-

Table 1. *Macaque Monkeys Used for Immunohistochemical Analysis*

Group	Survival in months (mean)	Age in years (mean)	Inoculum
SIV encephalitis (n = 12)	5–50 (12)	1–16 (5.8)	SIV
SIV without encephalitis (n = 6)	1–55 (19)	3–8 (3)	SIV
Uninfected (n = 6)	NA	1–6 (11.2)	–
EAE (n = 5)	1–2 (1.4)	4–9 (6.5)	hBWM

NA, not applicable; hBWM, human brain white matter.

Table 2. *Antibodies Used for Immunohistochemical Analysis*

Antigen	Subfamily	Antibody	Known cellular sources *in vivo*
MCP-1	C-C	3F11	MØ, endo, pericytes, fibro, epith, SM, keratinocytes, PMNs
MCP-2	C-C	2D5	PCs
MCP-3	C-C	6H5	MØ, endo, keratinocytes
MIP-1α	C-C	11A3	MØ, endo, fibro
MIP-1β	C-C	6E6	MØ, endo, fibro, keratinocytes
RANTES	C-C	9H9	Lym, keratinocytes
IP-10	C-X-C	4G3	MØ, endo, keratinocytes, fibro
IL-8	C-X-C	2A2	Lym, MØ, endo, PMNs, fibro, keratinocytes, epith

All antibodies were from LeukoSite, Inc. MØ, monocyte/macrophage; endo, endothelium; fibro, fibroblast; epith, epithelium; SM, smooth muscle; PCs, plasma cells; lym, lymphocyte; PMNs, neutrophils.

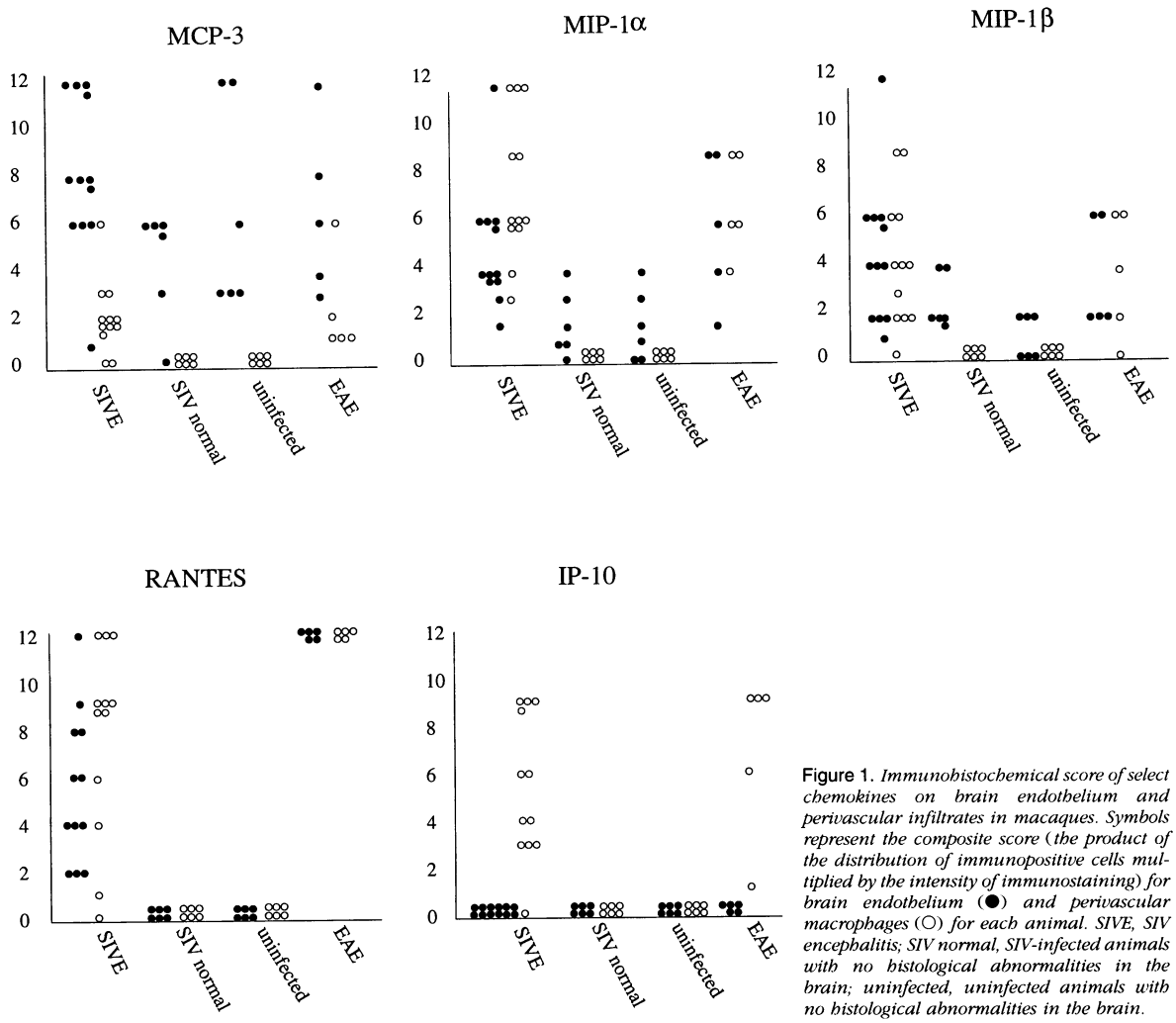


Figure 1. *Immunohistochemical score of select chemokines on brain endothelium and perivascular infiltrates in macaques. Symbols represent the composite score (the product of the distribution of immunopositive cells multiplied by the intensity of immunostaining) for brain endothelium (●) and perivascular macrophages (○) for each animal. SIVE, SIV encephalitis; SIV normal, SIV-infected animals with no histological abnormalities in the brain; uninfected, uninfected animals with no histological abnormalities in the brain.*

ducing EAE in the animals used in this study has been previously described in detail.[25]

Before use, all animals were negative for antibodies to SIV, type D retrovirus, and simian T-cell leukemia virus type 1. Animals were housed in accordance with standards of the American Association for Accreditation of Laboratory Animal Care. The investigators adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council.

Preparation of Monoclonal Antibodies

Monoclonal antibodies to human chemokines were produced by immunizing mice with 10 μg of recombinant chemokine (Peprotech, Rocky Hill, NJ) four to six times over 8 weeks. Splenocytes from immunized mice were fused with the SP2/0 cell line using standard procedures. The reactivity and specificity of the panel of anti-chemokine monoclonal antibodies are described elsewhere (C. Mackay, manuscript in preparation). Briefly, monoclonal antibodies to each chemokine were selected by screening by enzyme-linked immunosorbent assay as previously described.[26] The specificity of each monoclonal antibody was stringently controlled by enzyme-linked immunosorbent assay and Western blot assays with a large number of chemokines, including RANTES, MIP-1α, MIP-1β, eotaxin, MCP-1, MCP-2, MCP-3, IP-10, interleukin (IL)-8, GROα, and NAP-2. The antibodies used in this study had absolute specificity for the chemokines designated and in addition were selected because of their suitability for immunostaining on paraffin sections. In most cases, the monoclonal antibodies were also able to block ligand binding and/or chemotaxis of specific types of leukocytes.

Semiquantitative Immunohistochemical Analysis

Representative samples of brain were processed for histopathological and immunohistochemical examination. The monoclonal antibodies listed in Table 2 were used in a three-layer avidin-biotin-horseradish peroxidase complex procedure with diaminobenzidine as the chromogen.

Chemokine expression on endothelium and perivascular macrophages was semiquantitatively assessed by using intensity of immunostaining and distribution of positive cells as criteria. Each tissue was microscopically examined blindly by two observers and their scores averaged. The numerical values for the distribution of immunoreactive vessels and cellular infiltrates were as follows: 0, none; 1, focal; 2, multifocal; 3, diffuse. The scores for staining intensity were as follows: 0, none; 1, faint; 2, moderate; 3, marked; 4, intense. The product of these two values for distribution and intensity of positive vessels and cellular infiltrates (composite score) had a theoretical range of 0 to 12. These data are presented in Figure 1.

Results

Histopathological Examination of Macaque Monkeys

Eighteen of the twenty-nine macaques selected for this study were infected with SIV. Twelve of these eighteen SIV-infected macaques contained parenchymal and perivascular macrophage/microglial and multinucleate giant cell infiltrates characteristic of SIV encephalitis. Only one of the twelve animals with SIV encephalitis had a concurrent opportunistic infection in the CNS. This animal had *Toxoplasma gondii* tachyzoites and cysts within the cerebral cortex and spinal cord. Brains from the remaining six SIV-infected and from six normal, uninfected macaques were free of histopathological abnormalities.

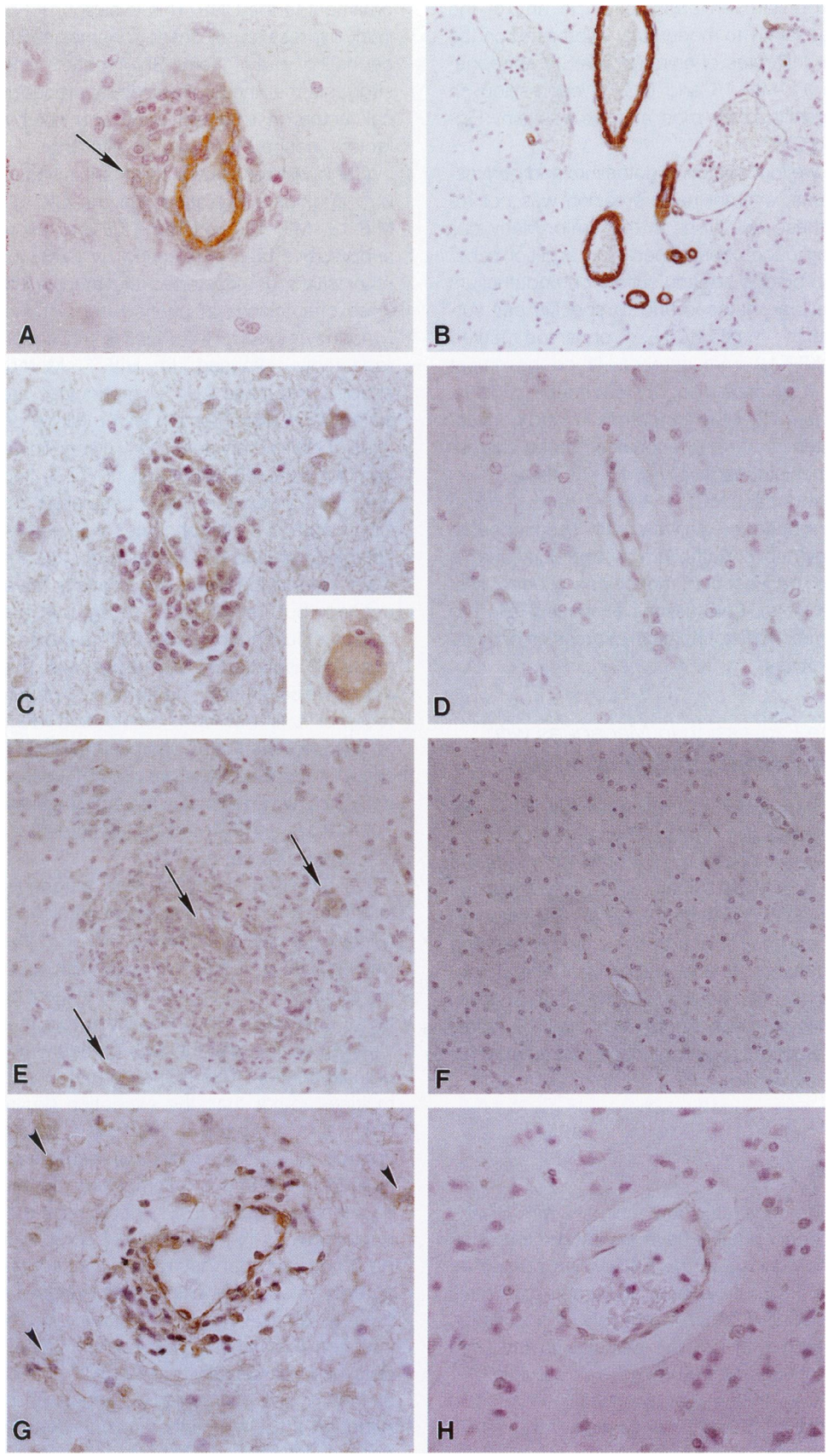
All five macaques with EAE had multifocal to extensive areas of inflammation characterized by central zones of necrosis and hemorrhage admixed with abundant neutrophils. The surrounding parenchyma contained dense aggregates of gitter cells and prominent vessels having variably sized perivascular cuffs of lymphocytes and macrophages.

MCP-3, MIP-1α, MIP-1β, RANTES, and IP-10 Are Elevated in Macaques with SIV Encephalitis

As the intimate interaction of circulating leukocytes and endothelium is pivotal for the development of cellular infiltrates in the CNS, we focused our examination of chemokine expression in SIV encephalitis on these two cell types. Endothelial expression of MCP-3, MIP-1α, MIP-1β, and RANTES was elevated in all macaques with SIV encephalitis (Figures 1 and 2, A, C, E, and G). MCP-3 expression on endothelium was intense and diffuse within meningeal vessels from both encephalitic and nonencephalitic animals (Figure 2B). However, endothelial expression of MCP-3 in parenchymal vessels was only markedly expressed in animals with SIV encephalitis (Figure 2A), but this expression was not uniformly associated with perivascular infiltrates. In animals with SIV encephalitis, capillaries and venules had moderate to intense expression of MIP-1α, MIP-1β, and RANTES (Figures 1 and 2, C, E, and G). Expression of MIP-1α, MIP-1β, and RANTES was seen in vessels surrounded by infiltrates but was also seen in vessels without infiltrates. In contrast to MCP-3, there was minimal endothelial expression of MIP-1α, MIP-1β, and RANTES in nonencephalitic animals (Figures 1 and 2, D, F, and H).

Perivascular monocytes/microglia and multinucleate giant cells in animals with SIV encephalitis expressed variable amounts of MCP-3, MIP-1α, MIP-1β, RANTES, and IP-10 (Figures 1 and 2, A, C, E, and G). This expression was not observed in nonencephalitic animals (Figure 2, B, D, F, and H). In general, MIP-1α and RANTES immunoreactivity on perivascular monocytes/microglia and multinucleate giant cells was moderate to intense and greater than that observed on the endothelium in animals with SIV encephalitis (Figures 1 and 2, C and G). IP-10, which

Figure 2. *Immunohistochemical expression of MCP-3, MIP-1α, MIP-1β and RANTES in SIV-infected macaques with* (A, C, E, *and* G) *and without* (B, D, F, *and* H) *SIV encephalitis. MCP-3 expression was intense on endothelium and smooth muscle in parenchymal vessels in animals with SIV encephalitis* (A), *but only faint expression was observed on perivascular infiltrates* (A, arrow). *MCP-3 expression on endothelium and smooth muscle in meningeal vessels was also intense in nonencephalitic animals* (B). *MIP-1α was intense on perivascular macrophages/microglia* (C) *and multinucleate giant cells* (C, inset) *in SIV-infected macaques with SIV encephalitis but negligible in nonencephalitic animals* (D). *MIP-1β immunoreactivity on CNS endothelium was intense* (E, arrows) *and moderate to marked on surrounding perivascular macrophages/microglia and multinucleate giant cells in SIV-infected macaques with SIV encephalitis* (E) *but faint to nonexistent in nonencephalitic animals* (F). *RANTES expression ranged from moderate to intense on endothelium and perivascular infiltrates in SIV-infected animals with encephalitis* (G) *but was negligible in nonencephalitic animals* (H). *Note faint to moderate expression on astrocytes* (G, arrowheads). *Avidin-biotin complex technique with Mayer's hematoxylin counterstain; original magnification, ×360* (A *to* D, G, *and* H) *and ×300* (E *and* F).


A
B
C
D
E
F
G
H

was not expressed on endothelium in any of the animals, was faintly to moderately expressed on the perivascular infiltrates of animals with SIV encephalitis (Figure 1). MIP-1β and MCP-3 expression on perivascular infiltrates in most animals was faint (Figure 1, A and E).

Although we focused on endothelium and perivascular infiltrates, chemokine expression was not restricted to these cell types in the brain. Many cell types can produce chemokines.[17] In addition, chemokines can bind to heparin sulfate on endothelium and extracellular matrices and to specific cell surface receptors.[17] In this study, we observed marked to intense MIP-1β immunoreactivity on basement membrane in animals with SIV encephalitis. Thus, many cell types other than endothelium and perivascular infiltrates in the CNS expressed these chemokines immunohistochemically (data not shown).

Despite abundant MCP-1, MCP-2, and IL-8 expression on macaque positive-control tissue (normal skin keratinocytes, endothelium of superficial dermal plexus, and perivascular monocytes, neutrophils, and plasma cells), CNS tissues examined failed to reveal any immunohistochemical expression of these three chemokines.

Chemokine Expression in Macaques with Experimental Allergic Encephalomyelitis

Chemokine expression in macaques with EAE was essentially identical to that in animals with SIV encephalitis (Figure 1) with the exception of moderate diffuse IL-8 and MCP-1 immunoreactivity (data not shown). Diffuse expression of MCP-1 was observed on neurons and astrocytes in the area surrounding dense inflammation. IL-8 was observed on endothelium and perivascular neutrophils and macrophages. As neutrophils were observed in all of the EAE cases, and IL-8 is a potent neutrophil chemoattractant, this finding was expected.

Discussion

This study demonstrates that animals with SIV-induced AIDS encephalitis have elevated expression of select chemokines in the CNS when compared with uninfected controls and SIV-infected animals without encephalitis. Most importantly, this elevation was associated with cellular infiltrates in the CNS and abundant virus as we have shown previously.[10] As these same chemokines were also elevated in animals with EAE, these findings likely represent a general phenomenon associated with leukocyte recruitment to the CNS. This is supported by the minimal expression of these chemokines in nonencephalitic brain from SIV-infected animals and suggests that these chemokines are induced by local factors in inflamed brain and not by systemic factors induced by infection with SIV.

Our *in vivo* results are important in light of recent *in vitro* findings suggesting that the C-C chemokines MIP-1α, MIP-1β, and RANTES are the major HIV-suppressive factors released by $CD8^+$ cells.[27] Another study demonstrated elevated levels of these three chemokines in purified populations of $CD4^+$ lymphocytes from HIV-negative individuals who are repeatedly exposed to HIV.[28] Moreover, they showed that these $CD4^+$ lymphocytes are more resistant to *in vitro* infection with multiple primary isolates of HIV-1 than were $CD4^+$ lymphocytes isolated from nonexposed individuals.[28] These findings suggest that, at least *in vitro*, chemokines released by $CD4^+$ and $CD8^+$ lymphocytes may have a substantial role in limiting HIV infection of cells. The mechanisms underlying these observations have not been defined, but Feng et al[29] report that a fusion coreceptor (fusin) along with CD4 enables T-cell-linetropic HIV isolates to infect target cells. This cofactor, a putative seven-transmembrane, G-protein-coupled receptor is similar (37% amino acid identity) to the receptor for the C-X-C chemokine IL-8.[29] Therefore, some chemokine receptors may function as fusion cofactors, which explains the antiviral activity of MIP-1α, MIP-1β, and RANTES in the studies of Cocchi et al[27] and Paxton et al.[28]

Our findings in SIV-infected macaques demonstrating elevated immunohistochemical expression of these same chemokines on endothelium and perivascular infiltrates in encephalitic brain containing abundant virus suggest that, at least in the brain, these chemokines do not play a role in containing viral replication and function primarily as mediators of inflammation. This is the normal function of chemokines rather than the exception (for review see Ref. 17). For instance, the role of chemokines in controlling leukocyte influx into tissues has been discussed in numerous inflammatory conditions such as atherosclerosis, EAE, rheumatoid arthritis, and pneumonia.[30–33] Furthermore, recent studies have demonstrated that neutralizing antibodies directed against select chemokines administered to rodents during inductive stages of inflammation significantly inhibited T cell and monocytic recruitment to sites of delayed hypersensitivity reaction,[34] pulmonary granulomas,[35] and interstitial pneumonia and fibrosis.[31] Although the interactions of chemokine expression and viral infection *in vivo* have not been investigated,

when knockout mice deficient for the gene encoding MIP-1α were exposed to Coxsackievirus, they maintained viral titers indistinguishable from those of wild-type mice but did not develop virus-induced myocarditis.[36] Thus, the primary function of chemokines *in vivo* may be as mediators of inflammation.

Although the exact mechanisms responsible for neurological damage associated with HIV infection are unknown, a correlation between macrophage/microglial infiltrates and clinical disease exists.[6] Exactly how macrophages/microglia contribute to HIV-associated dementia is unknown. However, their involvement is a unifying feature of all currently proposed mechanisms of neuronal dysfunction in patients with HIV-associated dementia.[37–39] The mechanisms responsible for development of these macrophage/microglial infiltrates remain a mystery. We have demonstrated elevated VCAM-1 in encephalitic brain from SIV-infected macaques and HIV-infected patients and that monocytic cells expressing $\alpha4\beta1$ (VLA-4) preferentially bind to these VCAM-1-expressing vessels.[15,23,40] However, it is unlikely that VCAM-1/$\alpha4\beta1$ interactions alone are responsible for recruitment of mononuclear cells to the CNS in HIV and SIV encephalitis.

Recently, studies examining brain from patients with HIV-associated dementia showed elevated MIP-1α and MIP-1β mRNA as compared with HIV-infected patients without dementia.[39] Likewise, we show elevated MIP-1α and MIP-1β in macaques with SIV encephalitis. MIP-1α and MIP-1β are potent chemoattractants for monocytes and lymphocytes[21,30] and in conjunction with cytokine-induced adhesion molecule expression provide a likely mechanism for monocyte recruitment to the CNS in HIV-infected patients.

Using immunohistochemical techniques, we are unable to differentiate between cells actively producing chemokines and cells binding released chemokines. However, the significant immunoreactivity on macrophages/microglia and multinucleate giant cells in animals with SIV encephalitis suggests that these cells are the primary producers of these chemokines. From previous studies we know that these macrophages/microglia are activated and contain abundant viral nucleic acid, antigen, and virus.[8,10] Furthermore, studies have shown that MIP-1α and MIP-1β are induced in cultured human monocytes upon infection with HIV.[39] This study suggests that, in addition to our previous findings demonstrating a role for VCAM-1 in monocyte recruitment to the CNS in macaques with SIV encephalitis, elevated C-C chemokines may be important contributors to this process.

Note Added in Proof

Since the submission of this manuscript it has been determined that MIP-1α, MIP-1β, and RANTES bind to C-C chemokine receptor 5, and that this is the major coreceptor for many macrophage-tropic strains of HIV-1.[41–45] The fusion coreceptor, termed LESTR/fusin, used by some T-cell-tropic strains of HIV-1 is the natural receptor for the C-X-C chemokine, stem cell-derived factor-1.[46,47]

Acknowledgments

We thank Drs. Ronald Desrosiers, Peter Dews, and Norman Letvin for use of tissue obtained from SIV-infected animals, Drs. Claude Genain and Stephen Hauser for use of tissues from macaques with EAE, and Alison Hampson for photographic assistance.

References

1. Price RW, Sidtis JJ, Brew BJ: AIDS dementia complex and HIV-1 infection: a view from the clinic. Brain Pathol 1991, 1:155–162
2. Wiley CA, Achim C: Human immunodeficiency virus encephalitis is the pathological correlate of dementia in acquired immunodeficiency syndrome. Ann Neurol 1994, 36:673–676
3. Masliah E, Ge N, Morey M, DeTeresa R, Terry RD, Wiley CA: Cortical dendritic pathology in human immunodeficiency virus encephalitis. Lab Invest 1992, 66:285–291
4. Everall IP, Luthert PJ, Lantos PL: Neuronal loss in the frontal cortex in HIV infection. Lancet 1991, 337:1119–1121
5. Asare E, Dunn G, Glass J, McArthur J, Luthert P, Lantos P, Everall I: Neuronal pattern correlates with the severity of human immunodeficiency virus-associated dementia complex. Am J Pathol 1996, 148:31–38
6. Glass JD, Fedor H, Wesselingh SL, McArthur JC: Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia. Ann Neurol 1995, 38:755–762
7. Lackner AA, Dandekar S, Gardner MB: Neurobiology of simian and feline immunodeficiency virus infections. Brain Pathol 1991, 1:201–212
8. Ringler DJ, Hunt RD, Desrosiers RC, Daniel MD, Chalifoux LV, King NW: Simian immunodeficiency virus-induced meningoencephalitis: natural history and retrospective study. Ann Neurol 1986, 23:S101–S107
9. Sharer LR, Baskin GB, Cho ES, Murphey-Corb M, Blumberg BM, Epstein LG: Comparison of simian immunodeficiency virus and human immunodeficiency virus encephalitides in the immature host. Ann Neurol 1988, 23:S108–S112
10. Lackner AA, Smith MO, Munn RJ, Martfeld DJ, Gardner

MB, Marx PA, Dandekar S: Localization of simian immunodeficiency virus in the central nervous system of rhesus monkeys. Am J Pathol 1991, 139:609–621
11. Nielsen SL, Petito CK, Urmacher CD, Posner JB: Subacute encephalitis in acquired immune deficiency syndrome: a postmortem study. Am J Clin Pathol 1984, 82:678–682
12. Persidsky Y, Nottet HSLM, Sasseville VG, Epstein LG, Gendelman HE: The development of animal model systems for HIV-1 encephalitis and associated dementia. J Neurovirol 1995, 1:229–243
13. Moses AV, Bloom FE, Pauza D, Nelson JA: Human immunodeficiency virus infection of human brain capillary endothelial cells occurs via a CD4/galactosylceramide-independent mechanism. Proc Natl Acad Sci USA 1993, 90:10474–10478
14. Blumberg BM, Gelbard HA, Epstein LG: HIV-1 infection of the developing nervous system: central role of astrocytes in pathogenesis. Virol Res 1994, 32:253–267
15. Nottet HSLM, Persidsky Y, Sasseville VG, Nakuna AN, Bock P, Zhai Q-H, Sharer LR, McComb RD, Swindells S, Soderland C, Gendelman HE: Mechanisms for the transendothelial migration of HIV-1-infected monocytes into brain. J Immunol 1996, 156:1284–1295
16. Springer TA: Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 1994, 76:301–314
17. Furie MB, Randolf GJ: Chemokines and tissue injury. Am J Pathol 1995, 146:1287–1301
18. Schall TJ, Bacon K, Camp RDR, Kaspari JW, Goeddel DV: Human macrophage inflammatory protein α (MIP-1α) and MIP-1β chemokines attract distinct populations of lymphocytes. J Exp Med 1993, 177:1821–1825
19. Loetscher P, Seitz M, Clark-Lewis I, Baggiolini M, Moser B: Monocyte chemotactic proteins MCP-1, MCP-2, and MCP-3 are major attractants for human $CD4^+$ and $CD8^+$ T lymphocytes. FASEB J 1994, 8:1055–1060
20. Rot A, Krieger M, Brunner T, Bischoff SC, Schall TJ, Dahinden CA: RANTES and macrophage inflammatory protein 1α induce the migration and activation of normal human eosinophil granulocytes. J Exp Med 1992, 176:1489–1495
21. Taub DD, Conlon K, Lloyd AR, Oppenheim JJ, Kelvin DJ: Preferential migration of activated $CD4^+$ and $CD8^+$ T cells in response to MIP-1α and MIP-1β. Science 1993, 260:355–358
22. Kestler H, Kodama T, Ringler D, Marthas M, Pedersen N, Lackner A, Regier D, Sehgal P, Daniel M, King N, Desrosiers R: Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. Science 1990, 239:586–591
23. Sasseville VG, Newman WA, Lackner AA, Smith MO, Lausen N, Beall D, Ringler DJ: Elevated vascular cell adhesion molecule-1 in AIDS encephalitis induced by simian immunodeficiency virus. Am J Pathol 1992, 141: 1021–1030
24. Desrosiers RC, Hansen-Moosa A, Mori K, Bouvier DP, King NW, Daniel MD, Ringler DJ: Macrophage-tropic variants of SIV are associated with specific AIDS-related lesions but are not essential for the development of AIDS. Am J Pathol 1991, 139:29–35
25. Massacesi L, Joshi N, Lee-Parritz D, Rombos A, Letvin NL, Hauser SL: Experimental allergic encephalomyelitis in cynomolgus monkeys. J Clin Invest 1992, 90:399–404
26. Ponath PD, Qin S, Ringler DJ, Clark-Lewis I, Wang J, Kassam N, Smith H, Shi X, Gonzalo J-A, Newman W, Glutlerraz-Ramos J-C, Mackay CR: Cloning of the human eosinophil chemoattractant, eotaxin: expression, receptor, and functional properties suggest a mechanism for the selective recruitment of eosinophils. J Clin Invest 1996, 97:604–612
27. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P: Identification of RANTES, MIP-1α, and MIP-1β as the major HIV-suppressive factors produced by $CD8^+$ T cells. Science 1995, 270:1811–1815
28. Paxton WA, Martin SR, Tse D, O'Brien TR, Skurnick J, VanDevanter NL, Padian N, Braun JF, Kotler DP, Wolinsky SM, Koup RA: Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposures. Nature Med 1996, 2:412–417
29. Feng Y, Broder CC, Kennedy PE, Berger EA: HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 1996, 272:872–877
30. Koch AE, Kunkel SL, Harlow LA, Mazarakis DD, Haines GK, Burdick MD, Pope RM, Strieter RM: Macrophage inflammatory protein-1α: a novel chemotactic cytokine for macrophages in rheumatoid arthritis. J Clin Invest 1994, 93:921–928
31. Smith RE, Strieter RM, Phan SH, Lukacs NW, Huffnagle GB, Wilke CA, Burdick MD, Lincoln P, Evanoff H, Kunkel SL: Production and function of murine macrophage inflammatory protein-1α in bleomycin-induced lung injury. J Immunol 1994, 153:4704–4712
32. Hulkower K, Brosnan CF, Aquino DA, Cammer W, Kulshrestha S, Guida MP, Rapoport DA, Berman JW: Expression of CSF-1, c-fms, and MCP-1 in the central nervous system of rats with experimental allergic encephalomyelitis. J Immunol 1993, 150:2525–2533
33. Yu X, Dluz S, Graves DT, Zhang L, Antoniades HN, Hollander W, Prusty S, Valente AJ, Schwartz CJ, Sonenshein GE: Elevated expression of monocyte chemoattractant protein 1 by vascular smooth muscle cells in hypercholesterolemic primates. Proc Natl Acad Sci USA 1992, 89:6953–6957
34. Rand ML, Warren JS, Mansour MK, Newman W, Ringler DJ: Inhibition of T cell recruitment and cutaneous delayed-type hypersensitivity-induced inflammation with antibodies to monocyte chemoattractant protein-1. Am J Pathol 1996, 148:855–864
35. Lukacs NW, Kunkel SL, Strieter RM, Warmington K, Chensue SW: The role of macrophage inflammatory protein 1α in *Schistosoma mansoni* egg-induced gran-

ulomatous inflammation. J Exp Med 1993, 177:1551–1559
36. Cook DN, Beck MA, Coffman TM, Kirby SL, Sheridan JF, Pragnell IB, Smithies O: Requirement of MIP-1α for an inflammatory response to viral infection. Science 1995, 269:1583–1585
37. Giulian D, Vaca K, Noonan CA: Secretion of neurotoxins by mononuclear phagocytes infected with HIV-1. Science 1990, 250:1593–1596
38. Genis P, Jett M, Bernton EW, Boyle T, Gelbard HA, Dzenko K, Keane RW, Resnick L, Mizrachi Y, Volsky DJ, Epstein LG, Gendelman HE: Cytokines and arachidonic metabolites produced during human immunodeficiency virus (HIV)-infected macrophage-astroglia interactions: implications for the neuropathogenesis of HIV disease. J Exp Med 1992, 176:1703–1718
39. Schmidtmayerova H, Nottet HSLM, Nuovo G, Raabe T, Flanagan CR, Dubrovsky L, Gendelman HE, Cerami A, Bukrinsky M, Sherry B: Human immunodeficiency virus type 1 infection alters chemokine β peptide expression in human monocytes: implications for recruitment of leukocytes into brain and lymph nodes. Proc Natl Acad Sci USA 1996, 93:700–704
40. Sasseville VG, Newman W, Brodie SJ, Hesterberg P, Pauley DR, Ringler DJ: Monocyte adhesion to endothelium in simian immunodeficiency virus-induced AIDS encephalitis is mediated by vascular cell adhesion molecule-1/α4β1 integrin interactions. Am J Pathol 1994, 144:27–40
41. Choe H, Farzam M, Sun Y, Sullivan N, Rollins B, Ponath P, Wu L, Mackay CR, LaRosa G, Newman W, Gerard N, Gerard C, Sodroski J: The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell 1996, 85:1135–1148
42. Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, Di Marzio P, Marmon S, Sutton RE, Hill CM, Davis CB, Peiper SC, Schall TJ, Littman DR, Landau NR: Identification of a major co-receptor for primary isolates of HIV-1. Nature 1996, 381:661–666
43. Doranz BJ, Rucker J, Yi Y, Smyth RJ, Samson M, Peiper SC, Parmentier M, Collman RG, Doms RW: A dual-tropic primary HIV-1 isolate that uses fusin and the B-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell 1996, 85:1149–1158
44. Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, Cayanan C, Maddon PJ, Koup RA, Moore JP, Paxton WA: HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. Nature 1996, 381:667–673
45. Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, Berger EA: CC CKR5: a RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 1996, 272: 1955–1958
46. Bleul CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, Sodroski J, Springer TA: The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV entry. Nature 1996, 382:829–833
47. Oberlin E, Amara A, Bachelerie F, Bessea C, Virelizier J-L, Arenzana-Seisdedos F, Schwartz O, Heard J-M, Clark-Lewis I, Legler DF, Loetscher M, Baggiolini M, Moser B: The CXC chemokine SDS-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature 1996, 282:833–835