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Short Communication

Elevated Vascular Cell Adhesion Molecule-1 in AIDS Encephalitis Induced by Simian Immunodeficiency Virus

Vito G. Sasseville,* Walter A. Newman,† Andrew A. Lackner,‡ Mary O. Smith,‖ Niels C. G. Lausen,* Dawson Beall† and Douglas J. Ringler*

From the Harvard Medical School, New England Regional Primate Research Center, Southborough, Massachusetts, Otsuka America Pharmaceutical, Inc.,† Rockville, Maryland, New Mexico State University,‡ New Mexico Regional Primate Research Laboratory, Holloman Air Force Base, New Mexico, and the University of California,‖ California Regional Primate Research Center, Davis, California*

AIDS encephalitis is a common sequela to HIV-1 infection in humans and simian immunodeficiency virus (SIVmac) infection in macaques. Although lentiviral-infected macrophages comprise parenchymal inflammatory infiltrates in affected brain tissue, the mechanisms responsible for leukocyte trafficking to the central nervous system in AIDS are unknown. In this study, we investigated the expression of various endothelial-derived leukocyte adhesion proteins in SIVmac-induced AIDS encephalitis. Encephalitic brains from SIVmac-infected macaques, but not uninflamed brains from other SIVmac-infected animals, were found to express abundant vascular cell adhesion molecule-1 (VCAM-1) protein on the majority of arteriolar, venular, and capillary endothelial cells. Soluble VCAM-1 concentrations in cerebrospinal fluid (CSF) from encephalitic animals were increased approximately 20-fold above those from animals without AIDS encephalitis. Expression of other endothelial-related adhesion molecules, including E-selectin, P-selectin, and intercellular adhesion molecule-1 (ICAM-1), was not uniformly associated with AIDS encephalitis. Thus, the presence of VCAM-1 in both brain and CSF was uniformly associated with SIVmac-induced disease of the central nervous system, and this expression may, at least in part, influence monocyte and lymphocyte recruitment to the central nervous system during the development of AIDS encephalitis. Moreover, measurement of soluble VCAM-1 in CSF may assist in the clinical assessment of animals or people with AIDS. (Am J Pathol 1992, 141:1021–1030)

One of the most devastating manifestations of HIV-1 infection involves an inflammatory disease of the central nervous system (CNS), referred to as AIDS encephalitis, AIDS encephalopathy, HIV encephalitis, or AIDS dementia complex.[1–3] This disorder is characterized by multifocal gliosis and parenchymal infiltrates of multinucleate giant cells and macrophages.[1,2,4] Many of the macrophages comprising these infiltrates contain HIV-1 protein, viral nucleic acid transcripts, and mature virions.[3,5–8] In addition, as these histologic changes are not associated with secondary opportunistic pathogens, it is believed that HIV encephalitis represents a primary lentiviral disease manifestation.

Not all patients infected with HIV-1 develop encephalitis. Approximately 50% of patients infected with HIV-1 have histologic evidence of AIDS encephalitis at the time of postmortem examination.[1,2,4,9] Similarly, approximately 50% of rhesus monkeys experimentally infected with simian immunodeficiency virus (SIVmac) develop an encephalitis similar to that seen in humans with

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Address reprint requests to Dr. Douglas J. Ringler, Harvard Medical School, New England Regional Primate Research Center, P.O. Box 9102, Southborough, MA 01772-9102.

AIDS.[10–12] SIVmac and HIV-1 share extensive sequence homology and have similar genomic organization, morphology, and biologic properties.[13–15] Most importantly, SIVmac-infected macaques develop immunodeficiency and a clinical syndrome identical in many ways to AIDS in humans. Thus, SIVmac-infected rhesus monkeys represent a useful model to study the pathogenesis of HIV encephalitis and other AIDS-related sequelae.

We have previously suggested that in simian AIDS, the presence of viruses *in vivo* with replicative potential for macrophages is essential, but not predictive, for the development of SIVmac-induced AIDS encephalitis.[16] Thus, both viral and host parameters are likely involved in determining which patients ultimately progress to lentiviral-induced disease of the CNS. Understanding the mechanisms responsible for the development of this disorder has obvious important consequences. First, therapeutic intervention studies can be directed to early cellular events before clinical disease. Secondly, less invasive means of diagnosis of encephalitis may be possible by detecting the earliest host parameters or pathogenetic mechanisms involved in leukocyte trafficking in the brain. Thirdly, a better understanding of the mechanisms involved in AIDS encephalitis will likely broaden our understanding of other primary lentiviral disease manifestations. Unfortunately, the sequence of events and mechanisms involved in the genesis of this disease remain largely unknown.

HIV- and SIVmac-induced encephalitis share many features in common with other inflammatory diseases. Leukocytes marginate and adhere to venular endothelium, extravasate from the vascular lumen, and eventually collect as perivascular and parenchymal inflammatory infiltrates. In a number of inflammatory processes, endothelial activation and *de novo* endothelial production of novel membrane proteins in the brain[17,18] and other tissues[19–23] precedes the formation of inflammatory infiltrates. Some of these endothelial membrane proteins are cell adhesion molecules, which have been shown to be involved in the adhesion of leukocytes to cytokine-stimulated endothelium *in vitro*.[19,24–28] Recent interest has focused on four proteins, designated E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1).[24,27,29–31] These molecules have been shown to differ in their leukocyte binding repertoire *in vitro* so that E-selectin selectively promotes endothelial adhesion to polymorphonuclear cells and memory T cells,[27,32] P-selectin enhances endothelial attachment to polymorphonuclear cells,[33] VCAM-1 mediates monocyte, lymphocyte, basophil, and eosinophil attachment,[19,24–26,34,35] whereas ICAM-1 promotes the adhesion of all types of leukocytes.[36,37] Differences in basal expression of these proteins exist, so that there is an absence, or at the most, little expression of E-selectin and VCAM-1 on resting endothelium.[19,20,28,38,39] In contrast, ICAM-1 and P-selectin are basally expressed on endothelium in many tissues.[17,31,36] Inasmuch as leukocyte-endothelial adhesion is requisite for vascular extravasation of leukocytes,[40] homing of specific leukocyte cell types to the CNS may be controlled by different endothelial expression of these leukocyte adhesion molecules.

In this study, we investigated the expression of leukocyte adhesion molecules in SIVmac-induced AIDS encephalitis. We found that encephalitic brains from SIVmac-infected animals expressed abundant VCAM-1 protein on the majority of parenchymal vessels. In addition, concentrations of soluble VCAM-1 (sVCAM-1) in cerebrospinal fluid (CSF) from encephalitic animals were markedly elevated over those from animals without AIDS encephalitis. These findings underscore the potential relevance of VCAM-1 expression in the pathogenesis of AIDS encephalitis. Furthermore, detection of elevated sVCAM-1 in CSF may assist in the clinical diagnosis of this manifestation in animals or people with AIDS.

Materials and Methods

Animals and Virus

Brain tissue from 37 rhesus monkeys (*Macaca mulatta*), one barbary macaque (*M. sylvana*), one pigtail macaque (*M. nemestrina*), and one cynomolgus monkey (*M. fascicularis*) was collected at death and used for immunohistochemical analysis. Thirty-four of these animals were experimentally infected with SIVmac before death. The strain of SIVmac used for experimental infection, survival time, age, and related animal data are contained within Table 1. The procedures associated with the experimental infection of many of these macaques with SIVmac have been described in detail previously.[14,16,41–45] Animals were inoculated either intravenously or intramuscularly with uncloned SIVmac251,[15,43,46] molecularly cloned SIVmac239,[14,47,48] or an uncloned macrophage-tropic variant of SIVmac239, termed SIVmac239/316[14,16] (Table 1). All animals were shown to be persistently infected with SIVmac by multiple viral isolations from peripheral blood mononuclear cells, as previously described,[46] and/or a rise in serum antibody response against SIVmac proteins.[49]

Normal brain tissue from five mature rhesus monkeys and one cynomolgus monkey was also evaluated as a control (Table 1). These monkeys were serologically negative for SIVmac before death.

In addition, CSF was collected from 30 SIVmac-infected rhesus monkeys at the time of death by lumbar or cisternal puncture. All 30 CSF samples were obtained

Table 1. *Macaques Used for Immunohistochemical Analysis*

Number of animals	Species	Survival range (days) (mean ± 1 SD)	Age range (years)* (mean ± 1 SD)	Inoculum
SIVmac encephalitis				
7	*M. mulatta*	64–1092 (488 ± 410)	1–10 (5.6 ± 3.1)	SIVmac251 (uncloned)
2	*M. mulatta*	167–808 (487)	3–4 (3.5)	SIVmac239 (molecularly cloned)
1	*M. sylvana*	818	Adult	SIVmac239 (molecularly cloned)
1	*M. nemestrina*	124	Adult	SIVmac239/316 (uncloned)
Nonspecific inflammatory CNS disease				
3	*M. mulatta*	56–201 (139 ± 61.3)	Adults	SIVmac251 (uncloned)
1	*M. mulatta*	785	12	SIVmac239/316 (uncloned)
Histologically normal brain				
17	*M. mulatta*	81–1438 (841 ± 363.4)	2–13 (4.7 ± 3.3)	SIVmac251 (uncloned)
2	*M. mulatta*	26–1024 (525)	Adults	SIVmac239 (molecularly cloned)
Normal control animals				
5	*M. mulatta*	N/A†	Adults	Uninfected
1	*M. fascicularis*	N/A†	12	Uninfected

* At death.
† Not applicable.

from animals persistently infected with uncloned SIVmac251,[15,43–46] molecularly cloned SIVmac239,[14,47,48] or SIVsm obtained from a captive sooty mangabey.[50] Five of these 30 animals are included in Table 1. An additional 28 CSF samples were collected from normal, uninfected rhesus monkeys by lumbar puncture. Serum was collected from representative select animals from all groups. All CSF and serum samples were stored at −80°C and thawed immediately before use.

All experimental animals were kept in accordance with the guidelines prepared by the Committee on the Care and Use of Laboratory Animals, National Research Council.

Immunohistochemistry

Representative samples of brain were processed for routine histopathologic and immunohistochemical examination. For immunohistochemistry, brain tissue was embedded in OCT compound (Miles Inc., Elkhart, IN) and snap-frozen in 2-methylbutane cooled in dry ice. Seven-micrometer tissue sections were cut on a cryomicrotome, mounted on gelatin-coated glass slides, and stored desiccated at −80°C until further use. Sections were fixed in 2% paraformaldehyde/0.5× PBS (pH 7.2) for 10 minutes at 4°C before performing immunoperoxidase procedures. A three-layer peroxidase-antiperoxidase (PAP) procedure with diaminobenzidine (DAB) as the chromogen was used, as previously described.[44,51,52] Single antigen labeling was performed using the monoclonal antibodies listed in Table 2.

Soluble VCAM-1 Assay

Levels of sVCAM-1 in CSF and serum were determined by a sandwich ELISA (Newman and Beall, manuscript in

Table 2. *Antibodies Used for Immunohistochemical Staining*

Antibody	Antigen	Reactivity	Source
2G7, 1E7	VCAM-1	Activated ECs, FDC, mononuclear phagocytes	W. Newman, Otsuka America Pharmaceutical, Inc., Rockville, MD
3B7	E-selectin	Activated ECs	W. Newman
S12	P-selectin	ECs, Platelets	Gift of R. McEver, Univ. of Oklahoma, Oklahoma City, OK
E1/7	ICAM-1	ECs, mononuclear leukocytes, FDC, FBs, Epithelial cells	Gift of M. Bevilacqua, University of San Diego, San Diego, CA
25.3	CD11a	Leukocytes (α chain of LFA-1)	AMAC Inc., Westbrook, ME
R1C7	SIVp27	SIV gag protein	Gift of M. Popovic and A. Minassian, NIH
EBM11	CD68	Mononuclear phagocytes	DAKO Corp., Carpinteria, CA

EC, endothelial cells; FDC, follicular dendritic cells; FB, fibroblasts.

preparation) using monoclonal antibodies recognizing non-overlapping epitopes of VCAM-1. Briefly, this assay was done by coating Nunc 96-well ELISA plates with 0.5 μg purified anti-VCAM-1 monoclonal antibody (clone 1E7) overnight at 4°C.[26] Plates were subsequently blocked for 1 hour with 1% bovine serum albumin at 37°C. Samples and standard controls were then incubated in coated wells overnight at 4°C. After washing, 0.05 μg biotinylated anti-VCAM-1 monoclonal antibody (clone 2G7)[26] was then added for 3 hours at 4°C, followed by 0.3 μg streptavidin peroxidase at 4°C for 30 minutes. O-phenylenediamine (1 mg/ml) in phosphate citrate buffer and 0.03% hydrogen peroxide were then added and gently shaken for 15 minutes in the dark. The reaction was stopped with 12.5% sulfuric acid and immediately read at an absorbance of 492 nm. For standard controls, purified sVCAM was obtained by ligation of a truncated form of VCAM-1 cDNA to the expression vector pCDN-1 and transfected into COS cells, as previously described.[53] The limit of detection of the assay was approximately 1.3 ng/ml sVCAM-1.

Results

Histopathologic Examination of SIVmac-infected Macaques

Of the 40 macaques selected for immunohistochemical analysis (Table 1), thirty-four were infected with SIVmac. Of these 34, eleven exhibited parenchymal macrophage and multinucleate giant cell infiltrates typical of SIVmac-induced AIDS encephalitis,[10,11] while 19 had no histopathologic abnormalities in the CNS (Table 1). Four had subtle atypical degenerative or inflammatory changes of the CNS characterized by slight perivascular lymphocytic cuffs or mild lymphocytic meningitis. Six brains were obtained from normal, uninfected macaques, and these tissues were free of histopathologic abnormalities.

Examination of brain tissue from the 30 SIVmac-infected animals from which CSF samples were obtained demonstrated that 7 had histologically confirmed AIDS encephalitis, 19 had no microscopic lesions, and 4 had nonspecific changes, including cerebral edema, slight mononuclear cell meningitis, and/or atypical inflammatory foci.

VCAM-1 Is Expressed on Endothelium in Animals with AIDS Encephalitis

Using standard immunohistochemical techniques[44,51,52] and a panel of monoclonal antibodies against cell adhesion molecules (Table 2), we examined frozen sections of brain from all study animals in Table 1. For the localization of VCAM-1, two monoclonal antibodies recognizing two distinct epitopes of VCAM-1[26] were used separately, and similar results were obtained with each reagent. We found that brain tissue from all 11 animals with SIVmac-induced AIDS encephalitis exhibited striking, diffuse distribution of VCAM-1 on arteriolar, venular, and capillary endothelium (Figure 1A, B). VCAM-1 expression was not restricted to inflammatory foci in these animals but was distributed to parenchymal vessels scattered throughout the entire brain (Figure 1B). However, VCAM-1 was most prevalent in white matter, where in 7 of the 11 cases, practically all vessels were immunoreactive. Interestingly, this predilection for VCAM-1 expression in white matter is consistent with previous findings describing AIDS encephalitis to be more severe in white matter regions.[9,11,12] Conversely, of the 19 SIVmac-infected animals without encephalitis, 17 had no detectable endothelial expression of VCAM-1, and the remaining 2 had only minimal expression to rare parenchymal vessels. In the six normal animals and the four SIVmac-infected animals with non-specific inflammatory changes, VCAM-1 expression on vascular endothelium was absent.

VCAM-1 Is Expressed on Ependyma and Mononuclear Inflammatory Cells in Animals with AIDS Encephalitis

In 6 of the 11 animals with encephalitis, sections of lateral and third ventricle were available for study, and in all 6, VCAM-1 was diffusely and markedly distributed on the luminal cell membrane of all ependymal lining cells (Figure 1C). Occasional CD11a+/CD68+ macrophages in the perivascular infiltrates from encephalitic brains also expressed VCAM-1 (Figure 1A). Brain tissue containing ependyma was available in 9 of the 19 SIVmac-infected animals without encephalitis. In contrast to the encephalitic group, in these nine cases, the localization of ependymal VCAM-1 was variable. Five of these animals had moderate diffuse immunoreactivity to ependymal cells, while minimal to no VCAM-1 was detected in the other four. Interestingly, in the 4 SIVmac-infected animals with atypical degenerative changes of the CNS, VCAM-1 expression was similar to that found in the group of 19 SIVmac-infected animals without encephalitis. Ependymal VCAM-1 expression in the six uninfected control animals was either minimal or not detected. Thus, from the immunohistochemical studies, there was a gradation of VCAM-1 expression on endothelium and ependyma, being most diffusely distributed in the SIVmac-infected animals with encephalitis. Fewer cells expressed detectable VCAM-1 in the other SIVmac-infected animals, including those with minor nonspecific inflammatory

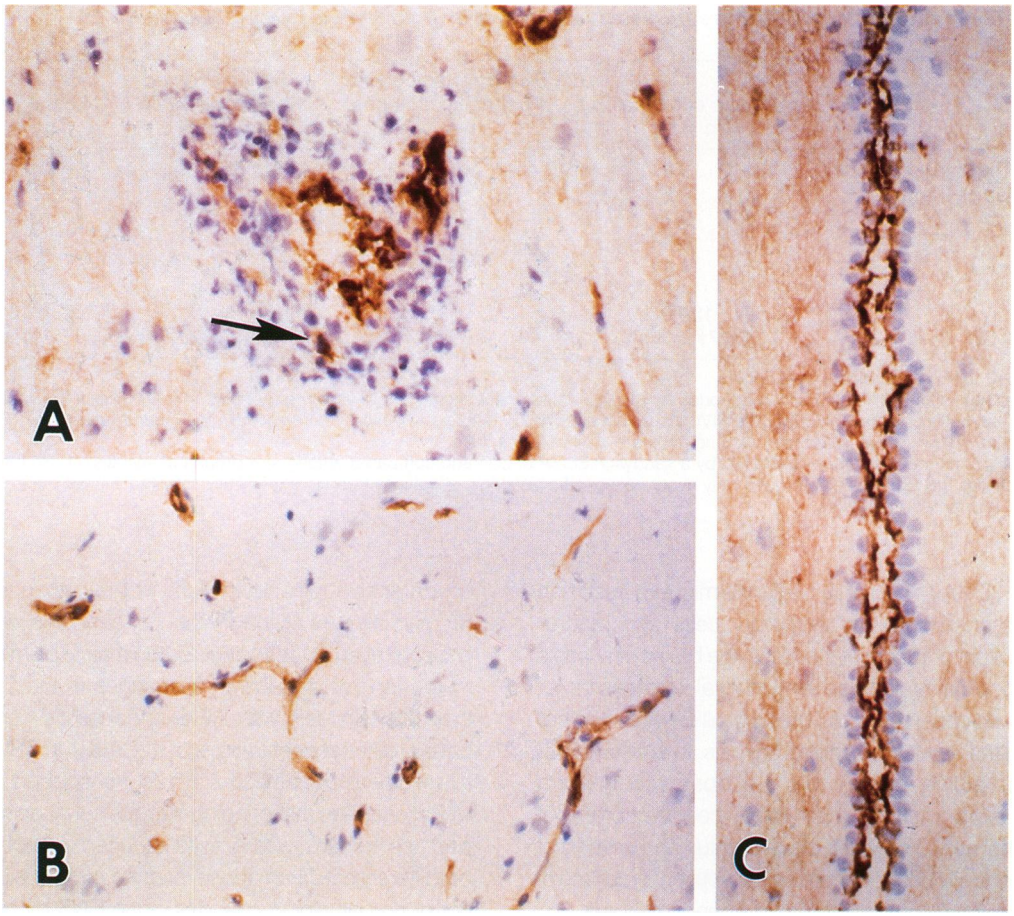

Figure 1. *Photomicrographs of VCAM-1 immunoreactivity in tissue sections of brain from animals with SIVmac-induced AIDS encephalitis. VCAM-1 expression on parenchymal endothelium is diffuse and is observed in areas of the brain with marked perivascular inflammatory infiltrates* (**A**) *as well as in areas with minimal inflammation* (**B**). *In these perivascular infiltrates, occasional mononuclear cells have VCAM-1 immunoreactivity* (**A**, *arrow*). *In addition, marked, diffuse localization of VCAM-1 is observed on the luminal aspect of ependymal lining cells* (**C**).

changes, whereas minimal to no expression of VCAM-1 was found in the uninfected control animals.

Concentrations of sVCAM-1 in CSF Are Increased in Animals with AIDS Encephalitis

Because our immunohistochemical studies demonstrated that VCAM-1 was intensely and consistently localized to ependyma and endothelium in brains from animals with SIVmac-induced AIDS encephalitis, we next examined levels of sVCAM-1 in frozen stored CSF and serum from SIVmac-infected and uninfected animals. Histopathologic examination and classification of brain tissue was made independently from the CSF analyses for sVCAM-1 concentrations. In normal rhesus macaques, 28 CSF samples were examined, and all contained ≤1.9 ng/ml of sVCAM-1 (Table 3). All CSF samples from SIVmac-infected animals without brain lesions also had low levels of sVCAM-1 (≤2.5 ng/ml; Table 3). Similarly, those samples from infected animals with nonspecific, atypical lesions of the CNS had comparably low concentrations of sVCAM-1 (Table 3). Conversely, animals with AIDS encephalitis had significantly greater quantities of sVCAM-1 in their CSF compared with the other three groups, ranging from 3.8 to 128.0 ng/ml (Kruskal-Wallis test, $P < 0.005$; Table 3). Quantification of sVCAM-1 in serum from normal and SIVmac-infected animals with and without encephalitis revealed a significant variability in sVCAM-1 concentrations, ranging from 65 to 1,285 ng/ml, and no significant difference between the levels obtained from each of the groups of animals was revealed (data not shown).

Expression of ICAM-1, E-selectin, and P-selectin Was Not Distinctive in AIDS Encephalitis

Unlike VCAM-1, the expression of ICAM-1 was not distinctive between the four groups of animals in this study.

Table 3. *Concentrations of sVCAM-1 Are Increased in CSF Samples from SIVmac-infected Macaques with AIDS Encephalitis.*

	SIVmac encephalitis (n = 7)	SIVmac-infected with nonspecific changes* (n = 4)	SIVmac-infected with normal brain (n = 19)	Normal, uninfected (n = 28)
sVCAM-1 (ng/ml) expressed as values or (range)	3.8 9.5 9.7 10.9 11.1 14.0 128.0	2.2 1.3‡ 1.3‡ 1.3‡	(1.3‡–2.5)	(1.3†–1.9)
Mean ± 1 SD	26.7† ± 41.4	1.5 ± 0.4	1.4 ± 0.3	1.4 ± 0.1

* See text for histopathologic description.

† Statistically different from other three groups by Kruskal-Wallis one-way analysis of variance by ranks[66]; $P < 0.005$. If the value of 128 is considered an outlier and not included, the SIVmac encephalitis group remains statistically different from the other three groups ($P < 0.005$).

‡ Samples below limit of detection (<1.3 ng/ml) were arbitrarily assigned a value of 1.3 ng/ml.

sVCAM-1 concentrations were measured by a sandwich ELISA in CSF and compared among four groups of animals: (1) SIVmac-infected with encephalitis, (2) SIVmac-infected with only minor nonspecific histologic alterations to brain, (3) SIVmac-infected without encephalitis, and (4) normal, uninfected macaques.

Although ICAM-1 was lightly, yet uniformly, expressed on solitary mononuclear inflammatory cells and macrophages within granulomatous foci in the brains of animals with encephalitis, diffuse endothelial expression of ICAM-1 was observed in the brains of all animals studied, including the uninfected control animals. In all tissue sections containing ependyma, we observed uniform ICAM-1 expression on the luminal cell membrane of ependymal cells. However, the SIV status and severity of CNS lesions did not influence the distribution or degree of immunoreactivity on endothelial or ependymal cells.

E-selectin was detected in brain tissue from two SIVmac-infected animals that died with terminal sepsis. One of these animals had AIDS encephalitis and the other did not. In these cases, E-selectin expression in the brain was limited to endothelium, and like that seen in other studies,[18,23,28,38] the presence of E-selectin was associated with a slight perivascular polymorphonuclear cell infiltrate. E-selectin was not present in brain from any of the other study animals.

In all four groups of animals examined, no immunohistochemical expression of P-selectin was detected on endothelial cells lining parenchymal vessels in the brain. However, P-selectin expression was observed on isolated meningeal vessels in all cases.

Discussion

This study demonstrates that animals with histologic evidence of SIVmac-induced AIDS encephalitis have significantly greater quantities of VCAM-1 in the CNS compared with other normal and other SIVmac-infected animals, whether assessed by localization using immunohistochemistry or quantification in the CSF. More specifically, SIVmac-infected animals with encephalitis have more diffuse and elaborate expression of VCAM-1 on parenchymal endothelium in brain than do either SIVmac-infected animals without encephalitis, normal animals, or even SIVmac-infected animals with mild inflammatory and/or degenerative changes in the CNS. Furthermore, animals with AIDS encephalitis consistently have greater concentrations of sVCAM-1 in CSF than do all the others, even those with mild, nonspecific histologic alterations. This latter finding suggests that animals, and perhaps humans, with lentiviral-induced encephalitis can be differentiated from those infected individuals with minor or no pathologic changes in the CNS by quantification of sVCAM-1 in CSF. As such, its measurement may provide a useful adjunct diagnostic tool for patients infected with HIV-1.

These results provide further associative evidence for the role of specific cell adhesion molecules in the cell composition of inflammatory infiltrates. Although widespread E-selectin expression has been observed in brains from cynomolgus monkeys experimentally infused with lethal doses of endotoxin,[18] in our study, it was not detected in any encephalitic animal uncomplicated by bacterial disease. Thus, changes in expression of E-selectin do not appear to be important in the evolution of simian AIDS encephalitis. Furthermore, despite *in vitro* evidence of increased ICAM-1 expression on cytokine stimulated endothelium,[36,37,54] variable endothelial basal expression observed in this study and in others[17,40] prevents assessing its role in simian AIDS encephalitis. Similarly, P-selectin is constitutively expressed in endothelium in multiple tissues.[55] However, because P-selectin was not constitutively expressed, or locally induced, in brain parenchymal vessels in our study animals, it is unlikely to have a role in leukocyte extravasation in simian AIDS encephalitis.

It has previously been shown that VCAM-1 mediates the adhesion of lymphocytes and monocytes to activated endothelium, and perhaps other cell types, through a leu-

kocyte integrin (VLA-4)-dependent pathway.[19,24,25,56,57] Since this study demonstrates that VCAM-1 is a predominant cell adhesion molecule found in the brains from encephalitic animals with AIDS, it is compelling that the inflammatory infiltrate in simian AIDS encephalitis is almost exclusively composed of macrophages and smaller number of lymphocytes.[11] However, it is unlikely that endothelial expression of VCAM-1 alone is sufficient to recruit mononuclear cells to the brains from these animals with AIDS, since a significant proportion of parenchymal vessels immunoreactive for VCAM-1 were not associated with adjacent inflammatory cell infiltrates. Although this finding can theoretically be explained by a difference in the temporal expression of VCAM-1 in relation to leukocyte extravasation from any one vessel, few marginating leukocytes were seen in these VCAM-1-expressing vessels. Thus, other criteria, such as decreases in vascular flow caliber, other chemotactic factors (i.e., monocyte chemoattractant factors)[58] and perhaps changes in the expression of other cell adhesion proteins (i.e. ICAM-1), may be essential for successful leukocyte extravasation to ensue. The putative role, if any, of ependymal VCAM-1 in mononuclear cell trafficking in AIDS encephalitis is less apparent, since ependymal VCAM-1 localization in SIVmac-infected animals without encephalitis was variable in intensity and distribution.

The expression of VCAM-1 on endothelial cells, like other cell adhesion molecules, is regulated, at least in part, by multiple microenvironmental influences. For example, IL-1, TNF-α, interferon-γ, and LPS have all been shown to increase adhesiveness of cultured endothelium for mononuclear and polymorphonuclear leukocytes.[27,59–63] At least part of this functional activity is mediated by *de novo* endothelial synthesis and membrane expression of VCAM-1.[24,26,30] Thus, microenvironmental changes in cytokine concentrations can have profound effects on endothelial biology and function. We have previously demonstrated that macrophages harvested from lungs of SIVmac-infected macaques have altered cytokine elaboration profiles upon LPS stimulation.[45] Hence, alterations in local or systemic concentrations of cytokines, resulting directly or indirectly from SIVmac infection in these animals, likely contribute to the changes in VCAM-1 production and distribution described in this report. Most notable among these is IL-4, which has recently been reported to induce VCAM-1-mediated binding of lymphocytes to endothelium, without simultaneously eliciting E-selectin or ICAM-1-mediated attachment.[64,65]

The source of elevated concentrations of sVCAM-1 in CSF from animals with SIVmac-induced AIDS encephalitis is not known. Although we were able to localize VCAM-1 to the luminal aspect of ependymal cells, this study does not demonstrate its production by these cells. Instead, through receptor-ligand interactions, sVCAM-1 from the CSF may be trapped on ependymal cells, thus accounting for its luminal distribution. If so, other potential sources of sVCAM-1 in the CSF would include plasma, epithelial or endothelial cells of the choroid plexus, and/or mononuclear cells in the CSF. In some instances, vascular permeability alterations in AIDS may be responsible for elevated sVCAM-1 in the CSF. In this regard, three of the seven encephalitic animals with elevated sVCAM-1 concentrations in the CSF were evaluated for CSF and serum albumin concentrations, and all three had elevated CSF albumin/serum albumin ratios (M. Smith, manuscript in preparation). However, 9 of the 19 animals in the SIVmac-infected group without encephalitis were also evaluated, and 2 of these 9 animals had elevated CSF albumin/serum albumin ratios. Thus, elevated sVCAM-1 in CSF is apparently more specific for SIVmac-induced AIDS encephalitis than albumin measurements alone.

In addition to investigations of the source of sVCAM-1 in the CSF of these SIVmac-infected macaques, the specificity of these findings should be established for AIDS encephalitis. It is not known if elevated sVCAM-1 concentrations in CSF are specific for AIDS encephalitis or if they represent manifestations secondary to profound inflammatory disease of the CNS. More specifically, to establish the overall significance of these findings, VCAM-1 expression should be examined in brain tissue or CSF in other clinically debilitating inflammatory disorders of the CNS. We did not find significantly increased expression or elaboration of VCAM-1 in tissue or in CSF respectively from animals with nonspecific inflammatory and/or degenerative changes of the CNS. However, other encephalitic processes of a more fulminant nature will be required for study. Nevertheless, the detection of increased concentrations of sVCAM-1 in CSF, even if not disease-specific, will assist in the overall clinical assessment of animals or people with lentiviral infections.

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