American Journal of Pathology, Vol. 144, No. 1, January 1994

Monocyte Adhesion to Endothelium in Simian Immunodeficiency Virus-Induced AIDS Encephalitis Is Mediated by Vascular Cell Adhesion Molecule-1/$\alpha 4\beta 1$ Integrin Interactions

Vito G. Sasseville,* Walter Newman,† Scott J. Brodie,* Paul Hesterberg,* Douglas Pauley,* and Douglas J. Ringler*

From the Department of Pathology, Harvard Medical School, New England Regional Primate Research Center, Southborough, Massachusetts, and Otsuka America Pharmaceutical, Inc.,† Rockville, Maryland*

Because the mechanisms associated with recruitment of monocytes to brain in AIDS encephalitis are unknown, we used tissues from rhesus monkeys infected with simian immunodeficiency virus (SIV) to examine the relative contributions of various adhesion pathways in mediating monocyte adhesion to endothelium from encephalitic brain. Using a modified Stamper and Woodruff tissue adhesion assay, we found that the human monocytic cell lines, THP-1 and U937, and the B cell line, Ramos, preferentially bound to brain vessels from monkeys with AIDS encephalitis. Using a combined tissue adhesion/immunohistochemistry approach, these cells only bound to vessels expressing vascular cell adhesion molecule-1 (VCAM-1). Furthermore, pretreatment of tissues with antibodies to VCAM-1 or cell lines with antibodies to VLA-4 (CD49d) inhibited adhesion by more than 70%. Intercellular adhesion molecule-1 (ICAM-1)/β2 integrin interactions were not significant in mediating cell adhesion to the vasculature in encephalitic simian brain using a cell line (JY) capable of binding rhesus monkey ICAM-1. In addition, selectin-mediated interactions did not significantly contribute to cell binding to encephalitic brain as there was no immunohistochemical expression of E-selectin and P-selectin in either normal or encephalitic brain, nor was there a demonstrable adhesive effect from L-selectin using L-selectin-transfected 300.19 cells on simian encephalitic brain. These results demonstrate that using the tissue adhesion assay, THP-1, U937, and Ramos cells bind to vessels in brain from animals with AIDS encephalitis using VCAM-1/α4β1 integrin interactions and suggest that VCAM-1 and VLA-4 may be integral for monocyte recruitment to the central nervous system during the development of AIDS encephalitis. (Am J Pathol 1994, 144:27–40)

Patients infected with HIV-1 manifest a plethora of diseases, including opportunistic infections, neoplasms, and inflammatory conditions. One of these inflammatory diseases, AIDS encephalitis, affects approximately 50% of patients with AIDS based on histological evaluation of the central nervous system (CNS) at the time of postmortem examination.[1–4] This disease is characterized by multifocal parenchymal and perivascular infiltrates of macrophages and multinucleated giant cells.[1,2,5,6] Because HIV-1 virions or gene products can be localized to macrophage infiltrates and microglia in affected brain tissue in the absence of secondary opportunistic pathogens,[1–3,7] AIDS encephalitis, similar to encephalitic disease induced by other animal lentiviruses, is likely a primary HIV-1-induced disorder. Furthermore, productive infection of brain macrophages or microglia is often associated with neurological disease, including motor, cognitive, and behavioral abnormalities.[2,8,9]

Supported by USPHS Awards AI29855, AI25644, RR00168, and 5T32RR07000 from the National Institutes of Health.

Current address of Drs. D. Ringler and W. Newman: LeukoSite, Inc., 800 Huntington Ave., Boston, MA 02115.

Accepted for publication September 3, 1993.

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.

However, the neuropathogenesis of AIDS encephalitis is not well understood. Because productive HIV-1 infection has been mostly confined to brain macrophage infiltrates, multinucleated cells, and microglia, without evidence of cytolytic infection of neurons, oligodendrocytes, or astrocytes, research on the pathogenetic mechanisms of this disorder has predominantly focused on the roles of viral or cell metabolites/toxins in neuronal dysfunction.[10–16] Inherent in these studies is the notion that HIV-1-infected brain macrophages or related cells either produce, or induce other CNS resident cells to produce factors that ultimately result in CNS injury. However, the inciting mechanisms responsible for initial macrophage/monocyte recruitment to brain during HIV-1 infection with subsequent development of macrophage infiltrates have not, to our knowledge, been described. Such information has important consequences because therapy could be directed at preventing monocyte/macrophage recruitment to the CNS in AIDS, thereby effectively reducing the subsequent deleterious effects of macrophage-associated toxic metabolites or lentiviral products.

It is now well established that inflammatory diseases, including those that involve the CNS, are characterized by a sequence of cell events that are, for the most part, initially staged at the vascular level. Leukocytes first roll and marginate and subsequently adhere to the endothelial membrane at inflammatory sites before extravasation from the vascular lumen. These cell events are mediated by a repertoire of cytokine-inducible, cell surface adhesion molecules on both endothelium and leukocytes. Three families of adhesion molecules have been described: selectins, integrins, and members of the immunoglobulin superfamily.[17–27] It is likely that the kinetics of expression and avidity of these adhesion molecules on both endothelium and leukocytes, in concert with chemotactic stimuli, are mechanisms that influence the numbers and types of inflammatory cells at tissue sites, since neutralization of adhesion molecule activity *in vivo* with relevant blocking antibodies influences the nature and extent of subsequent cellular infiltration.[28–31] Thus, determining which adhesion molecules are involved in a particular inflammatory disease has important implications for understanding events responsible for leukocyte recruitment and for subsequently designing rational therapeutic blocking strategies for that disease.

Similar to patients infected with HIV-1, rhesus monkeys infected with simian immunodeficiency virus (SIV) develop opportunistic infections, neoplasms, and primary lentivirus-induced inflammatory diseases, including AIDS encephalitis.[32–36] SIV exhibits extensive similarity to HIV-1 in genomic organization, gene sequences, and biological properties.[33,37,38] Furthermore, histological evaluations of AIDS encephalitis in both HIV-1-infected humans and SIV-infected monkeys have demonstrated significant morphological parallels.[39,40] Therefore, SIV-infected rhesus monkeys represent a useful animal model in which to study the pathogenesis of AIDS encephalitis in HIV-1-infected patients.

Previously, we reported that vascular cell adhesion molecule-1 (VCAM-1), a member of the immunoglobulin supergene family, was induced on brain endothelium in SIV-infected animals with AIDS encephalitis and that increased soluble levels of VCAM-1 could be detected in cerebrospinal fluid from affected animals.[41] The functional significance of this expression and the relative contribution of other adhesion molecules were not assessed. In this report, using a tissue-leukocyte adhesion assay and blocking antibodies to relevant adhesion ligands, we demonstrate that monocyte adhesion to endothelium in simian brain: 1) occurs in encephalitic tissue from SIV-infected animals and not in nonencephalitic brain, 2) is confined to endothelium expressing VCAM-1, and 3) is primarily mediated by the VCAM-1/VLA-4 adhesion pathway.

Materials and Methods

Animals and Virus

Brain tissue from 11 rhesus monkeys (*Macaca mulatta*) and one Formosan macaque (*Macaca cyclopis*) was collected at death and used for routine histological, immunohistochemical, and tissue adhesion assays. Six of these animals were experimentally infected with SIV. The strains of SIV used for experimental infection, survival time, age, and histopathological findings are shown in Table 1. The experimental inoculation of one of these animals, 316–85, has been described in detail previously.[33,42] Animals 144–85 and 121–84 were inoculated intravenously with 0.5 ml of a 1:100 dilution of cell-free supernatant from SIVmac251-infected human peripheral blood mononuclear cells. Animal 155–88 was infected intravenously with 1.0 ml of cell-free supernatant from CEMx174 cells infected with SIVmac239 (reverse transcriptase activity = 44×10^3 cpm/ml). Animals 108–89 and 118–89 were inoculated with SIV intracerebrally while under general anesthesia. Briefly, a small (2–3 cm) incision was made over the dorsolateral aspect of the calvarium, the dura exposed via trephination, and 0.9 ml of cell-free

Table 1. *SIV-Infected Rhesus Monkeys with AIDS Encephalitis*

Animal	Inoculum	Survival (days)	Age (yr)*	Other diagnoses†
155–88	SIVmac239 (molecularly cloned)	818	3	SV40 (kidney), GCP, CRY, GCL
144–85	SIVmac251 (uncloned)	264	>5	GCP, PC, CMV
316–85	SIVmac239 (molecularly cloned)	167	4	GCP
108–89	SIVsmmB670 (uncloned)	145	3	GCP, ADV
118–89	SIVsmmB670 (uncloned)	201	3	GCP, GCL
121–84	SIVmac251 (uncloned)	143	>8	GCP, GCL, PC, CRY

* At death.
† SV40, simian virus 40; GCP, multinucleated giant cell pneumonia; CRY, cryptosporidiosis; GCL, multinucleated giant cell lymphadenitis; PC, pneumocystis pneumonia; CMV, cytomegalovirus; ADV, adenovirus.

supernatant (16.4 ng of *gag* protein) from cultures of rhesus peripheral blood mononuclear cells infected with uncloned SIVDeltaB670 (kindly provided by Dr. Michael Murphey-Corb, Tulane University)[39,40,43] was injected into three separate cerebral sites immediately under the exposed dura. For certain experiments, brain tissue from an additional five SIVmac251- or SIVmac239-infected animals without histopathological evidence of AIDS encephalitis was used, as previously described in detail.[41] All animals demonstrated persistent infection with SIV by multiple viral isolations from peripheral blood mononuclear cells and/or a rise in serum antibody response against SIV proteins, as previously described.[44,45]

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.

Cells

The human monocytic cell lines, THP-1 and U937, and the human B cell line, Ramos, were obtained from the American Type Culture Collection (ATCC Nos. TIB 202, CRL 1593, and CRL 1596, Rockville, MD). The human JY B cell line was a gift from Dr. Jack Strominger (Harvard Medical School, Boston, MA). Parental murine 300.19 cells and L-selectin-expressing 300.19 cells transfected with human L-selectin cDNA[46,47] were the gifts of Dr. Thomas Tedder (Dana Farber Cancer Institute, Boston, MA). All cell lines were maintained in RPMI 1640 medium supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin, 2 mmol/L glutamine, 25 mmol/L HEPES, and 10% fetal calf serum (GIBCO Laboratories, Grand Island, NY). For the murine cells, medium was additionally supplemented with 10×10^{-5} mol/L 2-mercaptoethanol. Cells were cultured at a concentration 0.5×10^6/ml in 75-cm^2 polystyrene flasks (Corning Plastic Works, Corning, NY) in 5% CO_2 at 37 C. Immediately before use in the adhesion assay, cells were resuspended in complete medium at a concentration of 10^7 cells/ml. For immunocytochemical analyses, cells were cytocentrifuged onto glass slides, air-dried, and stored desiccated at −80 C until further use.

Rhesus Endothelial Cell Binding Assay

An adhesion assay utilizing rhesus monkey umbilical vein endothelial cells (RUVEC) was performed as a positive control for rhesus monkey intercellular adhesion molecule-1 (ICAM-1)-mediated adhesion of human cells to rhesus brain endothelium in the tissue adhesion assay. RUVEC were harvested from rhesus monkey umbilical cord, as previously described,[48] and were maintained in M199 medium (Whittaker Bioproducts, Walkersville, MD) containing 20% endotoxin-tested fetal bovine serum (GIBCO Laboratories), 90 μg/ml preservative-free porcine heparin (Sigma Chemical Co., St. Louis, MO), 100 μg/ml bovine hypothalamus extract (Otsuka America Pharmaceutical Inc., Rockville MD), 50 U/ml of penicillin, 50 μg/ml of streptomycin, and 0.25 μg/ml of amphotericin B (GIBCO Laboratories). Cells were grown to confluence in gelatin-coated 75-cm^2 polystyrene flasks and were passaged by dislodgment with washes of 2.5 g/l trypsin (Sigma Chemical Co.) and calcium- and magnesium-free Hanks' balanced salt solution (GIBCO Laboratories) and reseeding onto other gelatin-coated flasks or flat-bottomed 96-well plates.

For binding studies, RUVEC (<9th passage) were first plated onto gelatin-coated flat-bottomed 96-well plates and cultured for 48 hours or until confluent. Some cultures were subsequently stimulated with 10 ng/ml human recombinant tumor necrosis factor-α (TNF-α) (Sigma Chemical Co.) for 4 hours, followed by rinsing in supplemented medium. Next, 10^6 cells were added to each well and incubated with RUVEC for 30 minutes at 37 C. After incubation, nonadherent cells were removed from wells by

two consecutive washes with complete medium, placed on another 96-well plate, and counted. The number of nonadherent viable cells was determined by a colorimetric assay based on the ability of live cells to reduce a tetrazolium-based compound (MTT) (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) to a blue formazan product.[49] Briefly, 0.5 mg/ml of MTT was added to the nonadherent cell fraction for 4 hours at 37 C, during which mitochondrial dehydrogenase from the nonadherent cells reduced MTT, resulting in insoluble formazan crystals. The subsequent addition of 150 μl/well of anhydrous isopropanol yielded a colorimetric change proportionally related to the quantity of mitochondrial dehydrogenase and resultant formazan crystals. The number of nonadherent cells was then determined from a standard curve of optical density *versus* cell number, and the number of adherent cells was calculated by subtraction. Consistent results were obtained using RUVEC passaged less than 9 times.

Specificity for a particular adhesion pathway was deduced if significant inhibition of binding was achieved using relevant neutralizing antibodies against VCAM-1, LFA-1, VLA-4, or L-selectin (Table 2). Briefly, endothelial cell monolayers were incubated with $F(ab')_2$ fragments of antibodies, while cells were pretreated with relevant whole antibodies diluted in supplemented RPMI medium for 30 minutes at 37 C (Table 2). Control wells contained supplemented medium only or $F(ab')_2$ fragments of the antihuman transferrin receptor antibody, 5E9 (kindly provided by Drs. Walter Blattler and John Lambert, Immunogen Inc., Cambridge, MA), which does not recognize the rhesus monkey epitope (Drs. W. Blattler, J. Lambert, and D. Ringler, unpublished observations).[50] Anti-leukocyte common antigen (LCA; CD45) monoclonal antibodies (Table 2) (DAKO Corp., Carpinteria, CA) were used as control antibodies for cell lines.[51] Cell lines were added to wells containing RUVEC in the continued presence of antibodies and assayed as described.

Preparation of Brain Tissue

For histological analyses, representative sections of brain were fixed in 10% phosphate-buffered formalin, processed by routine histological techniques, and stained with hematoxylin and eosin. For immunohistochemistry and tissue adhesion assays, brain tissue was embedded in OCT compound (Miles Inc., Elkhart, IN) and snap-frozen in 2-methylbutane cooled in dry ice. Serial sections were cut on a cryomicrotome at a thickness of 7 μ for immunohistochemical analyses and 10 μ for tissue adhesion assays. Tissue sections were then mounted on gelatin-coated glass slides and allowed to air-dry at 25 C for 2 hours before use.

Tissue Adhesion Assay

A modification of the *in vitro* adhesion assay first described by Stamper and Woodruff[52] and used more recently by Yednock et al.[28] was utilized to assess cell adhesion to endothelium in encephalitic monkey brain. Desiccated sections of frozen brain from SIV-infected animals with and without encephalitis and uninfected normal control animals were first pretreated with RPMI 1640/10% fetal calf serum for 10 minutes at 4 C. Next, 10^6 cells in 100 μl of RPMI 1640/10% fetal calf serum were layered onto each tissue section, and the section and cells were placed on a gyratory shaker (Eberbach Corp., Ann Arbor, MI) at 60 rpm for 30 minutes at 4 C. For JY cell binding and evaluation of ICAM-1-mediated adhesion, the procedure was performed at 37 C. As a

Table 2. *Antibodies Used for Adhesion Assay*

Antibody	Antigen	Isotype	Reference No.	Source
2G7	VCAM-1	IgG1 $F(ab')_2$	53, 61	W. Newman, Otsuka America Pharmaceutical Inc., Rockville, MD
3B7	E-selectin	IgG2a $F(ab')_2$	53, 61	W. Newman
25.3	CD11a (α chain of LFA-1)	IgG1	78, 79	AMAC Inc., Westbrook, ME
MHM23	CD18	IgG1	80	DAKO Corp., Carpinteria, CA
HP2/1	CD49d (α chain of VLA-4)	IgG1	81, 82	AMAC Inc.
LAM1–3	L-selectin	IgG1	54	T. Tedder, Dana Farber Cancer Institute, Boston, MA
PD7/26 and 2B11	CD45	IgG1	51	DAKO Corp.
5E9	Human transferrin receptor (irrelevant in macaques)	IgG1 $F(ab')_2$	50	W. Blattler, J. Lambert, Immunogen Inc., Cambridge, MA

positive control for the evaluation of L-selectin-mediated adhesion, fresh frozen rhesus monkey lymph node sections were used as a binding substrate at 4 C. After incubation, all sections were gently rinsed in cold 0.15 mol/L phosphate-buffered saline (PBS), fixed in 1% glutaraldehyde in PBS for 15 minutes at 4 C, rinsed in PBS/0.2% gelatin (pH 7.2), and subsequently stained with 0.5% toluidine blue/30% ethanol for 15 to 20 seconds. After rinsing in 100% ethanol, the stained sections were mounted and examined microscopically. Experiments were performed a minimum of five times using encephalitic brain tissue from SIV-infected animals and three times on tissue from the uninfected controls.

To determine the relative contribution of various adhesion pathways in monocyte-endothelial interactions using this assay, we used a number of neutralizing monoclonal antibodies specifically directed at adhesion ligands on cell lines and on brain tissue from encephalitic and nonencephalitic SIV-infected animals as well as normal, uninfected control animals (Table 2). Briefly, before the cell-tissue incubation, tissue sections, cells, or both were incubated for 30 minutes at 4 C with saturating concentrations (20 μg/ml) of monoclonal antibodies to VCAM-1, the α4 subunit (CD49d) of VLA-4, and CD11a and CD18 of LFA-1 (Table 2). In addition, the specificity of anti-VCAM-1 antibody, 2G7, was assessed by saturating it with excess soluble VCAM-1 (100 μg/ml) for 30 minutes at 4 C before use in the tissue adhesion assay. Purified soluble VCAM-1 was obtained by ligation of a truncated form of VCAM-1 cDNA to the expression vector, pCDN-1, and transfected in COS cells as previously described.[53] Monoclonal antibodies to the human transferrin receptor (5E9; irrelevant in rhesus monkeys) and LCA were used on tissues and cell lines, respectively, as controls. L-selectin-expressing 300.19 cells and nontransfected 300.19 cells, along with neutralizing concentrations of LAM1–3 against L-selectin (ascitic fluid diluted 1:100)[54] were used in the assay to investigate the contribution of L-selectin in monocyte adhesion to simian brain endothelium.

Cell binding to tissue sections was assessed by microscopic examination and manual counting of bound cells per linear unit of endothelium. Briefly, quantification of cell binding was performed by evaluating all vessels in a tissue section having width and length sums (vascular dimension unit) ≥ 150 μ. For each vessel, a binding coefficient was determined by the quotient of bound cells/vascular dimension unit. At least 15 vessels in each tissue section were used to obtain the mean binding coefficient (MBC) ± 1 SEM for each tissue. MBC values could vary in absolute number between each assay; yet, relative to one case or another, there was significant consistency. Therefore, cell binding inhibition was measured as the percentage of MBC obtained using blocking or control antibodies relative to the MBC obtained from a serial tissue section in the same assay trial without antibody pretreatment.

Immunocytochemistry and Immunohistochemistry

For immunocytochemistry or immunohistochemistry, cytocentrifuged cells or tissue sections respectively were fixed in 2% paraformaldehyde/0.5X PBS (pH 7.2) for 10 minutes at 4 C. A three-layer peroxidase-antiperoxidase procedure or avidin-biotin-horseradish peroxidase complex (ABC) technique with diaminobenzidine as the chromogen was used, as previously described.[41,55–57] Cytocentrifuged cells were immunophenotypically characterized using monoclonal antibodies (Table 2) directed against LFA-1 (CD11a) and VLA-4 (CD49d), while tissue sections were labeled for VCAM-1, ICAM-1, E-selectin, and P-selectin, using monoclonal antibodies 2G7 (Table 2), E1/7 (courtesy of Dr. M. Bevilacqua, University of California at San Diego, San Diego, CA), 3B7 (Table 2), and S12 (courtesy of Dr. Rodger McEver, University of Oklahoma, Oklahoma City, OK), respectively.

Combined Tissue Adhesion Assay and Immunohistochemistry

To determine whether cell binding occurred on endothelium expressing VCAM-1, after performing the adhesion assay, select tissue sections were post-fixed in 0.5% glutaraldehyde, rather than 1.0% glutaraldehyde. An ABC technique was subsequently performed on the same tissue section using monoclonal antibody, 2G7, directed against VCAM-1. Immediately after chromogen development, these tissue sections were fixed again in 10% phosphate-buffered formalin for 10 minutes at 25 C, washed in PBS/0.2% gelatin, stained for 15 to 20 seconds in toluidine blue/30% ethanol, rinsed two times in 100% ethanol, and mounted.

Statistics

Significance was determined using the paired Student's *t*-test. Differences between means were considered significant when $P < 0.05$.

Results

Histopathological Examination of SIV-Infected Macaques

To investigate the selective adhesion of monocytic cells to encephalitic brain endothelium, brain tissue from six SIV-infected macaques was selected for this study (Table 1). On histopathological examination, brain tissue from all six had marked diffuse parenchymal and perivascular macrophage and multinucleated giant cell infiltrates, typical of SIV-induced AIDS encephalitis.[39,58] There was no microscopic evidence of opportunistic pathogens in the CNS from any animal. Various SIV-related diseases or opportunistic infections typical of SIV-infected macaques were seen in other organs, as described in detail elsewhere (Table 1).[32–36] Brain tissue from five SIV-infected rhesus monkeys without encephalitis and six normal uninfected macaques was used as control tissue. These tissues did not have any microscopic abnormalities.

Immunohistochemistry and Immunocytochemistry

Consistent with our previous report,[41] all six brains from animals with AIDS encephalitis expressed VCAM-1 on endothelium lining parenchymal vessels using immunohistochemistry. Conversely, brain tissue from the five SIV-infected animals without encephalitis and the uninfected control animals had no detectable endothelial expression of VCAM-1 in any section examined. ICAM-1 expression on vessels in encephalitic brain was not significantly different from the light, diffuse, constitutive expression observed in brains from nonencephalitic animals.[41] As described previously,[41] E- and P-selectin were not expressed in any affected or unaffected brain tissue. Macrophage infiltrates in encephalitic tissue expressed both CD11a (LFA-1) and CD49d (VLA-4) (Figure 1, A-C), leukocyte ligands for ICAM-1 and VCAM-1, respectively. Thus, immunohistochemical findings alone could not functionally differentiate the relative contributions of VCAM-1/VLA-4, ICAM-1/LFA-1, or L-selectin-mediated interactions in monocyte recruitment to CNS during the genesis of simian AIDS encephalitis.

Cells Selectively Adhered to Brain Endothelium from Animals with AIDS Encephalitis

We next used a tissue adhesion assay to discriminate the relative functional role of each adhesion pathway in monocyte binding to endothelium in simian encephalitic brain. Two monocytic cell lines (THP-1 and U937) and one B cell line (Ramos) were used to study VCAM-1/VLA-4 interactions, because all three cell lines express CD49d and have been shown to bind to VCAM-1-transfected COS cells or VCAM-1-expressing human umbilical vein endothelial cells (HUVEC).[59,60] Using this tissue adhesion assay, U937, THP-1, and Ramos cells selectively bound to endothelium in encephalitic brain tissue, while little to no binding was observed in normal macaque brain tissue (Table 3) or brains from nonencephalitic SIV-infected animals. MBC values for encephalitic brain tissue were generally one order of magnitude greater than MBCs for normal brain tissue, while Ramos cells did not bind at all to normal macaque brain (Table 3). Furthermore, cell binding was confined to endothelial sites (Figure 1D). However, there was no correlation between the severity of inflammatory changes in brain and the degree of cell adhesion to endothelium.

Cells Bound Only to Brain Endothelium Expressing VCAM-1

We next investigated whether cells adhered preferentially to specific endothelial immunophenotypes derived from brains with simian AIDS encephalitis. More specifically, immunohistochemistry was used after the tissue adhesion assay to assess if binding occurred only to vessels expressing specific adhesion molecules. Using this adhesion/immunohistochemistry combination, U937, THP-1, and Ramos cells bound only to vessels that had diffuse endothelial expression of VCAM-1 (Figure 1D). Vessels with either no or only subtle, focal VCAM-1 immunoreactivity did not have significant numbers of bound cells (Figure 1E).

VCAM-1/VLA-4 Mediated Significant Monocyte Attachment to Endothelium in Brain Tissue with SIV-Induced AIDS Encephalitis

Because cells in the tissue adhesion assay bound only to vessels expressing VCAM-1, we next examined the relative contribution of VCAM-1 in mediat-

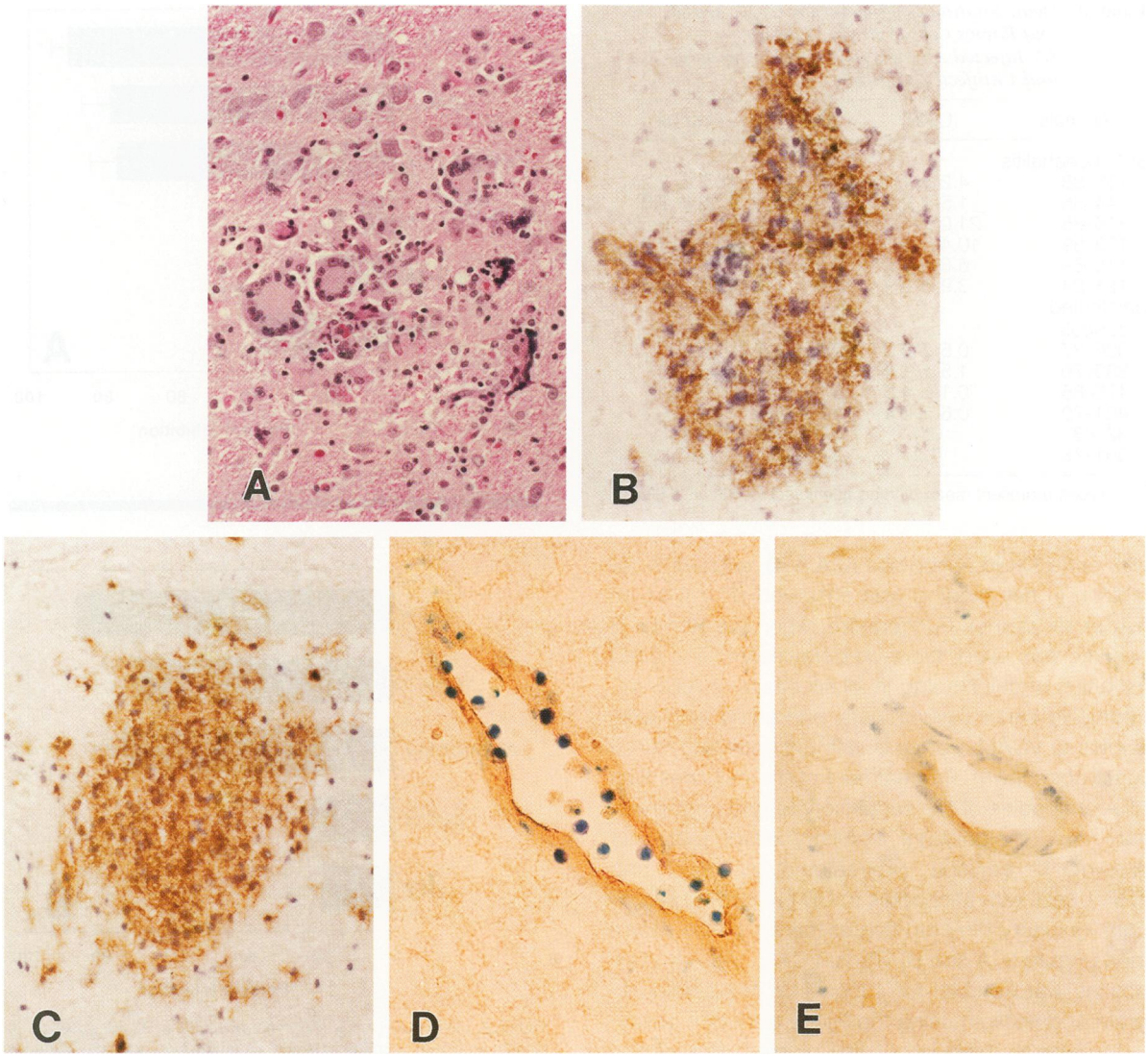

Figure 1. *Photomicrographs of brain from animals with SIV-induced AIDS encephalitis.* **A:** *Neuropil contains perivascular infiltrates of macrophages and multinucleated giant cells (H&E, ×175).* **B,C:** *Immunohistochemical staining demonstrates that many of these inflammatory cells co-express CD49d (VLA-4), the leukocyte ligand for endothelial VCAM-1* (**B**)*, and CD11a (LFA-1), the leukocyte ligand for ICAM-1* (**C**) *(ABC technique with Mayer's hematoxylin counterstain, ×230).* **D, E:** *On tissue sections from encephalitic brain, U937 cells adhere exclusively to vessels expressing VCAM-1, as detected by the brown diaminobenzidine reaction product* (**D**)*, whereas in the same assay, adjacent vessels with either subtle or no immunoreactivity for VCAM-1 did not have significant numbers of bound cells* (**E**) *(modified Stamper and Woodruff adhesion assay[52] and ABC technique with anti-VCAM-1 monoclonal antibody, 2G7; toluidine blue counterstain, ×350).*

ing binding of these cells to encephalitic brain endothelium. However, using U937 cells, pretreatment of affected tissues with whole antibodies directed at VCAM-1 (blocking antibody 2G7) resulted in a greater than 50% increase in cell binding relative to untreated brain tissue (data not shown). As previously observed,[61] these results were consistent with Fc-receptor-mediated binding of U937 cells to simian endothelium.

Therefore, we next pretreated encephalitic brain tissue sections with $F(ab')_2$ fragments of anti-VCAM-1 antibodies (2G7). Using either monocytic cell line, U937 (Figure 2A) or THP-1 (Figure 2B), in five of six affected brains, up to 70% inhibition of cell binding was observed after tissue pretreatment ($P < 0.05$). In contrast, pretreatment of the tissue sections with $F(ab')_2$ fragments of the control monoclonal antibody, 5E9, did not significantly inhibit cell binding in any of six cases with THP-1 cells and in five of six with U937 cells (Figure 2, A and B). When anti-VCAM-1 antibodies were preincubated with excess purified soluble VCAM-1 (100 μg/ml) before use in the tissue adhesion assay, the inhibitory effect of the antibodies on cell binding was abolished (Figure 2A). Similarly, when either U937 cells (Figure 2A) or THP-1 cells (Figure 2B) were pretreated

Table 3. *Mean Binding Coefficients for U937, THP-1, and Ramos Cells to Brain Vessels in SIV-Infected Animals with AIDS Encephalitis and Uninfected Controls*

Animals	U937	THP-1	Ramos
SIV encephalitis			
155–88	4.2 ± 1.1*	16.2 ± 2.3	15.7 ± 2.2
144–85	1.5 ± 0.8	12.9 ± 3.1	12.8 ± 2.8
316–85	21.0 ± 4.1	8.6 ± 1.3	27.7 ± 5.5
108–89	10.4 ± 2.0	28.0 ± 3.7	13.5 ± 2.3
118–89	6.6 ± 1.7	7.9 ± 1.5	17.8 ± 2.9
121–84	3.9 ± 0.9	17.9 ± 2.9	25.7 ± 4.4
Uninfected controls			
328–87	0.5 ± 0.3	2.3 ± 0.8	0.0
399–79	1.5 ± 1.4	1.4 ± 0.2	0.0
170–85	0.1 ± 1.5	5.2 ± 1.2	0.0
401–70	0.6 ± 0.4	1.2 ± 0.5	0.0
32–83	0.0	0.9 ± 0.5	0.0
501–78	0.0	2.4 ± 1.7	0.0

* Values represent mean binding coefficient ($\times 10^3$) ± 1 SEM.

with anti-CD49d antibodies (αVLA-4), up to 80% inhibition in cell binding was observed ($P < 0.01$ on five of six cases). Furthermore, when the Ramos B cell line was used, there was significant ($P < 0.05$) inhibition of cell binding in all six cases of AIDS encephalitis when tissues were pretreated with antibodies to VCAM-1 or cells with antibodies to VLA-4 (data not shown). Pretreatment of the cell lines with LCA antibodies of the same isotype and concentration had no significant inhibitory effect (data not shown). In addition, pretreatment of U937 and THP-1 cells with antibodies to CD11a had no significant inhibitory effect in five of the six cases examined (Figure 2, A and B). Thus, VCAM-1/VLA-4 interactions were crucial for successful cell binding to brain endothelium using the tissue adhesion assay.

In general, inhibition of cell binding was slightly greater using antibodies to CD49d rather than antibodies to VCAM-1 (Figure 2, A and B). Using U937 cells, anti-VCAM-1 antibodies inhibited cell binding 78 ± 8%, whereas anti-CD49d antibodies inhibited 89 ± 4% (Figure 2A). Similarly, using THP-1 cells, inhibition using anti-VCAM-1 antibodies was 71 ± 3%, while 87 ± 6% inhibition was obtained using antibodies to CD49d (Figure 2B).

ICAM-1/LFA-1 Interactions at Endothelial Sites in Encephalitic Brain Tissue Were Not Significant Using the Tissue Adhesion Assay

Because we could not demonstrate ICAM-1/CD11a-mediated adhesion between U937 or THP-1 cells and endothelium in brain from animals with AIDS encephalitis, we next examined ICAM-1-mediated

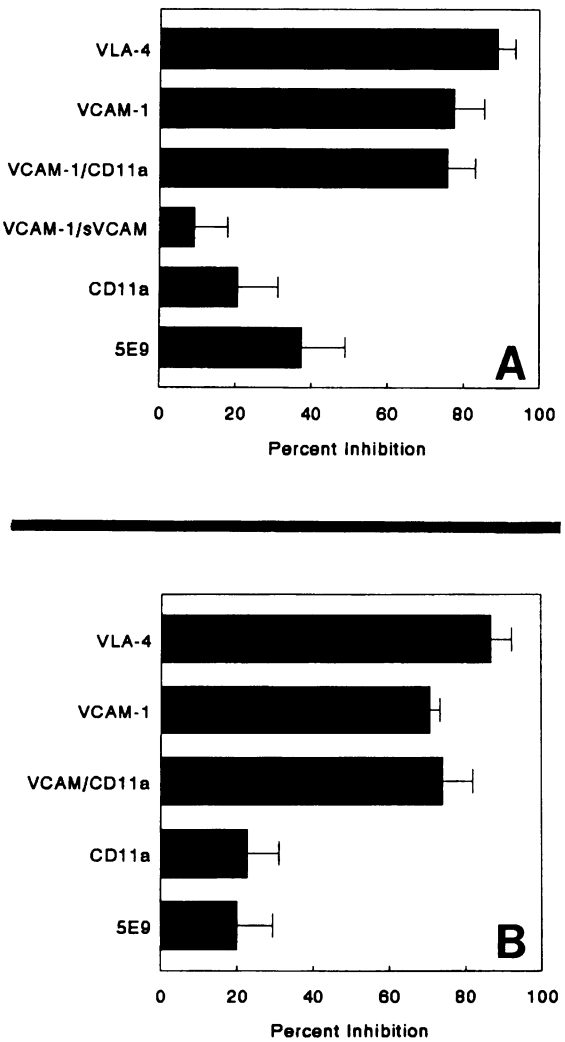

Figure 2. *Inhibition (%) of U937 (A) and THP-1 (B) cell adhesion to vessels in frozen sections of brain from animals with SIV-induced AIDS encephalitis when neutralizing antibodies against VLA-4 (CD49d) and LFA-1 (CD11a) were used to pretreat cells or $F(ab')_2$ fragments of antibodies against VCAM-1, irrelevant $F(ab')_2$ fragments (5E9), or soluble VCAM-1 (sVCAM) were used to pretreat brain tissue sections. Each bar represents the mean ± 1 SEM for all six cases of encephalitis, and inhibition for each case was assessed in relation to binding without pretreatment.*

interactions further using the B lymphoblastoid cell line, JY, which has previously shown to avidly adhere to unstimulated HUVEC using the LFA-1/ICAM-1 pathway.[59,62] Similarly, when assayed on RUVEC, JY cells bound unstimulated monolayers when performed at 37 C (Figure 3). In addition, pretreatment of JY cells with neutralizing concentrations of monoclonal antibodies directed against either CD11a or CD18 significantly decreased this binding by 40% and 55%, respectively ($P < 0.02$; Figure 3). These results demonstrated that JY cells avidly bound RUVEC, at least in part, using the LFA-1/ICAM-1 pathway and, most importantly, could

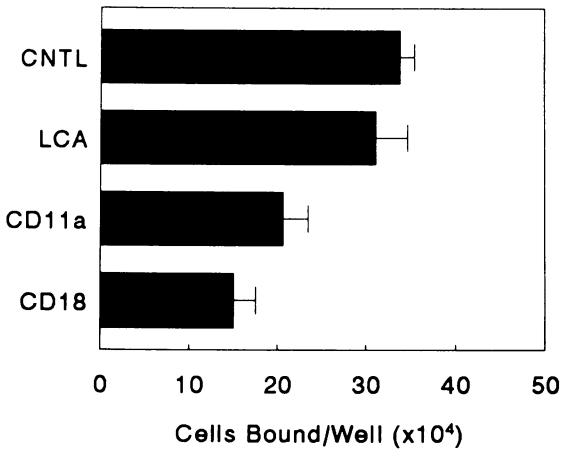

Figure 3. *Number of adherent JY cells to wells containing unstimulated RUVEC monolayers. Antibodies to CD11a, CD18, and LCA were used to pretreat JY cells before addition to wells. Untreated bound JY cells are represented by the CNTL bar. Each bar represents the mean number of bound cells to RUVEC in triplicate wells ± 1 SEM.*

be used to assess ICAM-1-mediated leukocyte adherence to endothelium in simian encephalitic brain.

However, using the tissue adhesion assay at 37 C, we observed that JY cells bound only minimally to brain endothelium in animals with AIDS encephalitis (Table 4). Binding coefficients using JY cells were generally one order of magnitude lower than those obtained using U937, THP-1, or Ramos cells (Table 3). Moreover, pretreatment of JY cells with antibodies to CD18 had no significant effect on cell binding in four of five cases examined (Table 4). In contrast, pretreatment of JY cells with antibodies against VLA-4 decreased cell binding by greater than 85%, although this decrease was statistically significant in only three of five cases due to the overall low MBCs observed in the cases examined. Therefore, using JY cells in the tissue adhesion assay, ICAM-1/β2 integrin-mediated adhesion was not observed in the majority of encephalitic cases examined. These results are concordant with the low level, yet detectable, expression of ICAM-1 on endothelium in both encephalitic and normal brain using immunohistochemical techniques.

L-Selectin Had Only a Modest Contribution in Cell Adhesion to Endothelium in Simian AIDS Encephalitis

We next examined the relative functional role of the selectins in cell attachment to endothelium in simian encephalitic brain using the tissue adhesion assay. P- and E-selectin were not considered further, since no detectable expression of either one was seen in encephalitic brain using immunohistochemistry.[41] However, since monocyte attachment to activated HUVEC is partially mediated by L-selectin under nonstatic conditions,[63] we examined the contribution of L-selectin in mediating cell binding to endothelium in encephalitic tissue.

As a positive control, we first utilized L-selectin-expressing 300.19 cells transfected with human L-selectin cDNA on rhesus monkey lymph node sections and found that these cells bound to high endothelial venules and germinal centers, similar to previous studies using human lymph node substrates.[46] When these cells were applied to encephalitic brain in the adhesion assay, there was a moderate decrease in cell binding in all five cases examined after pretreatment of cells with neutralizing antibodies to L-selectin; however, in only one case (118–89) was this decrease statistically significant (Table 5). Furthermore, when binding coefficients between L-selectin-transfected and nontransfected 300.19 cells were compared, no significant increase in binding to encephalitic brain was observed with the L-selectin transfected cell line (Table 5). These results demonstrate that, at least using the tissue adhesion assay, the contribution of L-selectin in mediating cell adhesion in simian AIDS encephalitis, if any, appears to be minor compared with VCAM-1/VLA-4 interactions.

Discussion

This study demonstrates that the monocytic cell lines U937, THP-1, and Ramos selectively adhere to endothelium in brain from animals with SIV-induced

Table 4. *Mean Binding Coefficients for JY Cell Binding to Brain Vessels in SIV-Infected Animals with AIDS Encephalitis (Modified Stamper and Woodruff Assay at 37 C)*

Animals	CNTL*	αLCA	αCD18	αCD49d
155–88	1.4 ± 0.6†	0.15 ± 0.1‡	0 ± 0‡	0.23 ± 0.2‡
144–85	1.5 ± 0.9	0.5 ± 0.5	0.78 ± 0.2	0 ± 0
108–89	4.5 ± 2.2	2.69 ± 1.3	1.38 ± 0.6	0.63 ± 0.4
118–89	5.2 ± 1.5	3.46 ± 1.7	5.18 ± 1.7	0.99 ± 0.5‡
121–84	13.5 ± 7.2	5.16 ± 2.4	6.2 ± 3.6	1.05 ± 0.7‡

* CNTL: control, no antibody pretreatment.
† Values represent mean binding coefficient ($\times 10^3$) ± 1 SEM.
‡ Statistically significant ($P < 0.05$) from CNTL group.

Table 5. *Mean Binding Coefficients for L-Selectin-Transfected 300.19 and Nontransfected 300.19 Cells to Brain Vessels in SIV-Infected Animals with AIDS Encephalitis*

	300.19 L-selectin-transfected		300.19 nontransfected	
Animals	αLCA	αL-sel	αLCA	αL-sel
155–88	1.6 ± 0.5*	0.4 ± 0.3	6.4 ± 1.1	3.5 ± 0.9
144–85	3.5 ± 1.5	0.4 ± 0.4	3.7 ± 2.2	3.8 ± 1.4
108–89	3.8 ± 1.4	2.8 ± 1.4	3.1 ± 0.6	1.7 ± 0.8
118–89	7.5 ± 1.3	2.9 ± 0.9†	27.1 ± 3.3	19.9 ± 1.8
121–84	1.7 ± 1.1	0.3 ± 0.3	0.9 ± 0.5	2.7 ± 1.7

* Values represent mean binding coefficient ($\times 10^3$) ± 1 SEM.
† Statistically significant ($P < 0.05$) from αLCA group.

AIDS encephalitis. Furthermore, up to 80% of this adhesion can be inhibited by pretreatment of cells or tissues with neutralizing monoclonal antibodies to either VCAM-1 or VLA-4. In contrast, using cells capable of interacting with rhesus monkey ICAM-1 and the ligand for L-selectin, we found no similar functional role for ICAM-1- and L-selectin-mediated adhesion in simian encephalitic brain. Furthermore, endothelial E- and P-selectin were not detected in encephalitic parenchymal tissue using immunohistochemistry. These findings suggest that the monocytic cell infiltrates characteristic of AIDS encephalitis may be recruited as a result of VLA-4/VCAM-1-mediated adhesive interactions between blood monocytes and brain endothelium. As such, inhibition of this pathway may prove effective in decreasing monocyte recruitment to the CNS in lentivirus-infected animals or humans.

In all cases except one, pretreatment of cells with neutralizing antibodies to VLA-4 (αCD49d) resulted in slightly greater inhibition of cell binding than did pretreatment of tissue sections with neutralizing antibodies to VCAM-1. These differences were statistically significant ($P < 0.02$) for THP-1 cells but not for U937 cells. Although speculative, the greater efficiency of antibodies to VLA-4 in cell binding inhibition compared with antibodies to VCAM-1 alone may be the result of their ability to prevent cell interactions with both VCAM-1 and the CS1 domain of fibronectin.[24,64] Alternatively, the anti-VLA-4 antibody may be a more effective blocking reagent. Whether neutralization of VLA-4 activity *in vivo* results in a more efficient blockade of VLA-4/VCAM-1 interactions than that obtained by neutralization of VCAM-1 activity alone will require further studies with animal inflammatory models.

These findings provide further evidence for the selective recruitment of mononuclear cells to inflammatory sites by the activity of specific adhesion molecules. Most recently, Yednock et al.[28] and Baron et al.[29] have reported VLA-4-dependent adhesion of lymphocytes to activated endothelium in CNS during the development of experimental autoimmune encephalomyelitis. Moreover, VCAM-1/VLA-4 interactions have been implicated in the development of mononuclear cell infiltrates in adjuvant-induced[30] and spontaneous arthritis,[21,23,24,65] atherogenesis,[66] experimental models of dermatitis,[30,31,67,68] and in murine cardiac allograft rejection.[69]

Recently, monocyte attachment to TNF-α-stimulated HUVEC under nonstatic conditions was shown to be mediated, in part, by L-selectin.[63] However, we observed only minimal L-selectin-mediated binding of L-selectin-transfected 300.19 cells to vessels in encephalitic brain, despite their avidity for rhesus monkey high endothelial venules in normal lymph node. In addition, significant inhibition of binding using neutralizing antibodies to L-selectin was observed in only one of five cases examined. Moreover, adhesion between L-selectin-transfected 300.19 cells and endothelium in simian AIDS encephalitic tissue was quantitatively similar on a per case basis to binding using nontransfected 300.19 cells. Therefore, when examined individually in the adhesion assay, the contribution of L-selectin-mediated binding, if any, appears modest.

Similarly, in five of the six cases examined, we did not observe significant inhibition of cell binding by pretreatment of U937 and THP-1 monocytic cells with neutralizing antibodies to CD11a (LFA-1), the leukocyte ligand for endothelial ICAM-1. These results are similar to findings in rat brain with experimental autoimmune encephalitis in which U937 cells and rat peripheral blood mononuclear cells were not inhibited from binding to encephalitic brain after pretreatment with antibodies to LFA-1.[28] Moreover, using a cell line (JY) that avidly binds to rhesus monkey ICAM-1 at 37 C, we observed no significant inhibition of cell binding to encephalitic tissue after pretreatment of cells with antibodies to CD18 in four of five cases examined. Therefore, similar to the findings for L-selectin, ICAM-1/β2 integrin interactions, at least in the tissue adhesion assay, appear less critical in cell binding to endothelium in SIV-induced AIDS encephalitis than do VCAM-1/α4β1 integrin binding events.

First described by Stamper and Woodruff,[52] adhesion assays on tissue sections have been useful in delineating specific endothelial/leukocyte interactions at the tissue level in both normal and diseased states.[24,28,70] Collectively, with immunohistochemical detection of specific adhesion proteins in tissues,

these assays provide useful functional data for further work on novel anti-inflammatory therapeutic approaches. As no immunohistochemical detection of P-selectin was observed in either normal or encephalitic brain,[41] it is unlikely to have a role in monocyte adhesion to endothelium during the development of simian AIDS encephalitis. Similarly, E-selectin was not observed in any case of simian AIDS encephalitis not complicated by terminal sepsis.[41] However, it is unlikely that VCAM-1/VLA-4 interactions are solely responsible for recruitment of mononuclear cells to the CNS in SIV-induced AIDS encephalitis, and we cannot rule out that other adhesion pathways, although having a minor contribution in the adhesion assay when examined individually, work in concert *in vivo* to significantly contribute to monocyte/endothelial adhesion.

Several investigators have demonstrated that AIDS encephalitis is characterized by the increased expression or elaboration of specific cytokines, such as TNF-α[71] and TGF-β,[72] or toxic metabolites, such as quinolinic acid,[73] in the brain. More recently, Genis et al.[10] reported that cocultures of HIV-infected monocytes and astroglia produce high levels of TNF-α and IL-1β. Interestingly, both of these cytokines induce endothelial expression of VCAM-1 *in vitro*.[17,59,61] Furthermore, cells expressing VLA-4 selectively bind to TNF-α-stimulated[74,75] and IL-1-stimulated[76,77] endothelium *in vitro*. However, the exact mechanisms responsible for VCAM-1 expression and subsequent monocyte recruitment to the CNS in AIDS encephalitis are unknown. Regardless of the inciting events, abrogation of monocyte recruitment in this disorder, using neutralization strategies against VLA-4 or VCAM-1 activity *in vivo*, may be a useful therapeutic approach for either patients with AIDS encephalitis or those HIV-1-infected individuals predisposed to it.

Acknowledgments

The authors thank Dr. Thomas F. Tedder for his generous gifts of L-selectin-transfected 300.19 cells and monoclonal antibody, LAM1–3, and for his advice and critical review of the manuscript; Dr. Jack Strominger for JY cells; Drs. W. Blattler, J. Lambert, M. Bevilacqua, and R. McEver for their generous contributions of monoclonal antibodies; Dr. M. Murphey-Corb for SIVDeltaB670; Drs. Ronald Desrosiers, Muthiah Daniel, and Peter Dews for use of tissue obtained from study animals; Edie Estabrook for manuscript preparation; and June Armstrong and Alison Hampson for photographic assistance.

References

1. 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
2. Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC, Cleary P: The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. Science 1988, 239:586–591
3. Wiley CA, Schrier RD, Nelson JA, Lampert PW, Oldstone MBA: Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. Proc Natl Acad Sci USA 1986, 83:7089–7093
4. Anders KH, Guerra WF, Tomiyasu U, Verity MA, Vinters HV: The neuropathology of AIDS. Am J Pathol 1986, 124:537–558
5. Epstein LG, Sharer LR, Goudsmit J: Neurological and neuropathological features of human immunodeficiency virus infection in children. Ann Neurol 1988, 23: S19-S23
6. Vazeux R, Brousse N, Jarry A, Henin D, Marche C, Vedrenne C, Mikol J, Wolff M, Michon C, Rozenbaum W, Bureau J-F, Montagnier L, Brahic M: AIDS subacute encephalitis: identification of HIV-infected cells. Am J Pathol 1987, 126:403–410
7. Koenig S, Gendelman HE, Orenstein JM, Dal Canto MC, Pezeshkpour GH, Yungbluth M, Janotta F, Aksamit A, Martin MA, Fauci AS: Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science 1986, 233:1089–1093
8. Michaels J, Sharer LR, Epstein LG: Human immunodeficiency virus type 1 (HIV-1) infection of the nervous system: a review. Immunodefic Rev 1988, 1:71–104
9. Price RW, Sidtis J, Rosenblum M: The AIDS dementia complex: some current questions. Ann Neurol 1988, 23:S27-S33
10. 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
11. Giulian D, Vaca K, Noonan CA: Secretion of neurotoxins by mononuclear phagocytes infected with HIV-1. Science 1990, 250:1593–1596
12. Bernton E, Bryant H, Decoster M, Orenstein JM, Ribas J, Meltzer MS, Gendelman HE: No direct neuronotoxicity by HIV-1 virions or culture fluids from HIV-1-infected T cells or monocytes. AIDS Res Hum Retroviruses 1992, 8:495
13. Dreyer EB, Kaiser PK, Offermann JT, Lipton SA: HIV-1 coat protein neurotoxicity prevented by calcium channel antagonists. Science 1990, 248:364–367

14. Sabatier J-M, Vives E, Mabrouk K, Benjouad A, Rochat H, Duval A, Hue B, Bahraoui E: Evidence for neurotoxic activity of *tat* from human immunodeficiency virus type 1. J Virol 1991, 65:961–967
15. Pulliam L, Herndier BG, Tang NM, McGrath MS: Human immunodeficiency virus-infected macrophages produce soluble factors that cause histological and neurochemical alterations in cultured human brains. J Clin Invest 1991, 87:503–512
16. Brenneman DE, Westbrook GL, Fitzgerald SP, Ennist DL, Elkins KL, Ruff MR, Pert CB: Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. Nature 1988, 335:639–642
17. Rice GE, Munro JM, Bevilacqua MP: Inducible cell adhesion molecule 110 (INCAM-110) is an endothelial receptor for lymphocytes: a CD11/CD18-independent adhesion mechanism. J Exp Med 1990, 171:1369–1374
18. Rice GE, Munro JM, Corless C, Bevilacqua MP: Vascular and nonvascular expression of INCAM-110: a target for mononuclear leukocyte adhesion in normal and inflamed human tissues. Am J Pathol 1991, 138: 385–393
19. Lewis RE, Buchsbaum M, Whitaker D, Murphy GF: Intercellular adhesion molecule expression in the evolving human cutaneous delayed hypersensitivity reaction. J Invest Dermatol 1989, 93:672–677
20. Sobel RA, Mitchell ME, Fondren G: Intercellular adhesion molecule-1 (ICAM-1) in cellular immune reactions in the human central nervous system. Am J Pathol 1990, 136:1309–1316
21. Koch AE, Burrows JC, Haines GK, Carlos TM, Harlan JM, Leibovich SJ: Immunolocalization of endothelial and leukocyte adhesion molecules in human rheumatoid and osteoarthritic synovial tissues. Lab Invest 1991, 64:313–320
22. van der Wal AC, Das PK, Tigges AJ, Becker AE: Adhesion molecules on the endothelium and mononuclear cells in human atherosclerotic lesions. Am J Pathol 1992, 141:1427–1433
23. Postigo AA, Garcia-Vicuña R, Diaz-Gonzalez F, Arroyo AG, De Landázuri MO, Chi-Rosso G, Lobb RR, Laffon A, Sánchez-Madrid F: Increased binding of synovial T lymphocytes from rheumatoid arthritis to endothelial-leukocyte adhesion molecule-1 (ELAM-1) and vascular cell adhesion molecule-1 (VCAM-1). J Clin Invest 1992, 89:1445–1452
24. van Dinther-Janssen ACHM, Horst E, Koopman G, Newmann W, Scheper RJ, Meijer CJLM, Pals ST: The VLA-4/VCAM-1 pathway is involved in lymphocyte adhesion to endothelium in rheumatoid synovium. J Immunol 1991, 147:4207–4210
25. Lasky LA: Selectins: interpreters of cell-specific carbohydrate information during inflammation. Science 1992, 258:964–969
26. Bevilacqua MP, Nelson RM: Selectins. J Clin Invest 1993, 91:379–387
27. Spertini O, Luscinskas FW, Kansas GS, Munro JM, Griffin JD, Gimbrone MA, Jr, Tedder TF: Leukocyte adhesion molecule-1 (LAM-1, L-selectin) interacts with an inducible endothelial cell ligand to support leukocyte adhesion. J Immunol 1991, 147:2565–2573
28. Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N: Prevention of experimental autoimmune encephalomyelitis by antibodies against $\alpha 4\beta 1$ integrin. Nature 1992, 356:63–66
29. Baron JL, Madri JA, Ruddle NH, Hashim G, Janeway CA, Jr: Surface expression of $\alpha 4$ integrin by CD4 T cells is required for their entry into brain parenchyma. J Exp Med 1993, 177:57–68
30. Issekutz TB, Issekutz AC: T lymphocyte migration to arthritic joints and dermal inflammation in the rat: differing migration patterns and the involvement of VLA-4. Clin Immunol Immunopathol 1991, 61:436–447
31. Issekutz TB: Inhibition of *in vivo* lymphocyte migration to inflammation and homing to lymphoid tissues by the TA-2 monoclonal antibody: a likely role for VLA-4 *in vivo*. J Immunol 1991, 147:4178–4184
32. Letvin NL, Daniel MD, Sehgal PK, Desrosiers RC, Hunt RD, Waldron LM, MacKey JJ, Schmidt DK, Chalifoux LV, King NW: Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. Science 1985, 230:71–73
33. 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, 248:1109–1112
34. McClure HM, Anderson DC, Fultz PN, Ansari AA, Lockwood E, Brodie A: Spectrum of disease in macaque monkeys chronically infected with SIV/SMM. Vet Immunol Immunopathol 1989, 21:13–24
35. Baskin GB, Murphey-Corb M, Watson EA, Martin LN: Necropsy findings in rhesus monkeys experimentally infected with cultured simian immunodeficiency virus (SIV)/delta. Vet Pathol 1988, 25:456–467
36. Simon MA, Chalifoux LV, Ringler DJ: Pathologic features of SIV-induced disease and the association of macrophage infection with disease evolution. AIDS Res Hum Retroviruses 1992, 8:327–337
37. Gardner MB, Luciw PA: Simian immunodeficiency viruses and their relationship to the human immunodeficiency viruses. AIDS 1988, 2:S3-S10
38. Desrosiers RC: The simian immunodeficiency viruses. Annu Rev Immunol 1990, 8:557–578
39. Sharer LR, Baskin GB, Cho E-S, 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
40. Sharer LR, Michaels J, Murphey-Corb M, Hu F-S, Kuebler DJ, Martin LN, Baskin GB: Serial pathogenesis study of SIV brain infection. J Med Primatol 1991, 20: 211–217

41. Sasseville VG, Newman WA, Lackner AA, Smith MO, Lausen NCG, 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
42. 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
43. Murphey-Corb M, Martin LN, Rangan SRS, Baskin GB, Gormus BJ, Wolf RH, Andes WA, West M, Montelaro RC: Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. Nature 1986, 321:435–437
44. Daniel MD, Letvin NL, King NW, Kannagi M, Sehgal PK, Hunt RD: Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. Science 1985, 228:1201–1204
45. Daniel MD, Letvin NL, Sehgal PK, Schmidt DK, Silva DP, Solomon KR, Hodi FS, Jr, Ringler DJ, Hunt RD, King NW, Desrosiers RC: Prevalence of antibodies to 3 retroviruses in a captive colony of macaque monkeys. Int J Cancer 1988, 41:601–608
46. Kansas GS, Ley K, Munro JM, Tedder TF: Regulation of leukocyte rolling and adhesion to high endothelial venules through the cytoplasmic domain of L-selectin. J Exp Med 1993, 177:833–838
47. Tedder TF, Penta AC, Levine HB, Freedman AS: Expression of the human leukocyte adhesion molecule, LAM1: identity with the TQ1 and Leu-8 differentiation antigens. J Immunol 1990, 144:532–540
48. Jaffe EA, Nachman RL, Becker CG, Minick CR: Culture of human endothelial cells derived from umbilical veins: identification by morphologic and immunologic criteria. J Clin Invest 1973, 52:2745–2756
49. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB: Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 1987, 47:936–942
50. Scott CF, Jr, Goldmacher VS, Lambert JM, Jackson JV, McIntyre GD: An immunotoxin composed of a monoclonal antitransferrin receptor antibody linked by a disulfide bond to the ribosome-inactivating protein gelonin: potent *in vitro* and *in vivo* effects against human tumors. J Natl Cancer Inst 1987, 79:1163–1172
51. Warnke RA, Gatter KC, Falini B, Hildreth P, Woolston R-E, Pulford K, Cordell JL, Cohen B, De Wolf-Peeters C, Mason DY: Diagnosis of human lymphoma with monoclonal antileukocyte antibodies. N Engl J Med 1983, 309:1275–1281
52. Stamper HB, Jr, Woodruff JJ: Lymphocyte homing into lymph nodes: *in vitro* demonstration of the selective affinity of recirculating lymphocytes for high-endothelial venules. J Exp Med 1976, 144:828–833
53. Polte T, Newman W, Raghunathan G, Gopal TV: Structural and functional studies of full-length vascular cell adhesion molecule-1: internal duplication and homology to several adhesion proteins. DNA Cell Biol 1991, 10:349–357
54. Spertini O, Kansas GS, Reimann KA, MacKay CR, Tedder TF: Function and evolutionary conservation of distinct epitopes on the leukocyte adhesion molecule-1 (TQ-1, Leu-8) that regulate leukocyte migration. J Immunol 1991, 147:942–949
55. Ringler DJ, Wyand MS, Walsh DG, MacKey JJ, Chalifoux LV, Popovic M, Minassian AA, Sehgal PK, Daniel MD, Desrosiers RC, King NW: Cellular localization of simian immunodeficiency virus in lymphoid tissues. 1. Immunohistochemistry and electron microscopy. Am J Pathol 1989, 134:373–383
56. Ringler DJ, Hancock WW, King NW, Letvin NL, Daniel MD, Desrosiers RC, Murphy GF: Immunophenotypic characterization of the cutaneous exanthem of SIV-infected rhesus monkeys: apposition of degenerative Langerhans cells and cytotoxic lymphocytes during the development of acquired immunodeficiency syndrome. Am J Pathol 1987, 126:199–207
57. Horvath CJ, Simon MA, Bergsagel DJ, Pauley DR, King NW, Garcea RL, Ringler DJ: Simian virus 40-induced disease in rhesus monkeys with simian acquired immunodeficiency syndrome. Am J Pathol 1992, 140:1431–1440
58. Ringler DJ, Hunt RD, Desrosiers RC, Daniel MD, Chalifoux LV, King NW: Simian immunodeficiency virus-induced meningoencephalitis: natural history and retrospective study. Ann Neurol 1988, 23:S101-S107
59. Osborn L, Hession C, Tizard R, Vassallo C, Luhowskyj S, Chi-Rosso G, Lobb R: Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. Cell 1989, 59:1203–1211
60. Osborn L, Vassallo C, Benjamin CD: Activated endothelium binds lymphocytes through a novel binding site in the alternately spliced domain of vascular cell adhesion molecule-1. J Exp Med 1992, 176:99–107
61. Graber N, Gopal TV, Wilson D, Beall LD, Polte T, Newman W: T cells bind to cytokine-activated endothelial cells via a novel, inducible sialoglycoprotein and endothelial leukocyte adhesion molecule-1. J Immunol 1990, 145:819–830
62. Dustin ML, Springer TA: Lymphocyte function-associated antigen-1 (LFA-1) interaction with intercellular adhesion molecule-1 (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. J Cell Biol 1988, 107:321–331
63. Spertini O, Luscinskas FW, Gimbrone MA, Jr, Tedder TF: Monocyte attachment to activated human vascular endothelium *in vitro* is mediated by leukocyte adhesion molecule-1 (L-selectin) under nonstatic conditions. J Exp Med 1992, 175:1789–1792
64. Elices MJ, Osborn L, Takada Y, Crouse C, Luhowskyj S, Hemler ME, Lobb RR: VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a

site distinct from the VLA-4/fibronectin binding site. Cell 1990, 60:577–584
65. Wilkinson LS, Edwards JCW, Poston RN, Haskard DO: Expression of vascular cell adhesion molecule-1 in normal and inflamed synovium. Lab Invest 1993, 68:82–88
66. Cybulsky MI, Gimbrone MA, Jr: Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 1991, 251:788–791
67. Briscoe DM, Cotran RS, Pober JS: Effects of tumor necrosis factor, lipopolysaccharide, and IL-4 on the expression of vascular cell adhesion molecule-1 *in vivo*: correlation with $CD3^+$ T cell infiltration. J Immunol 1992, 149:2954–2960
68. Silber A, Reimann K, Hendricks E, Mercier M, Newman W, Ringler D: Kinetic expression of endothelial adhesion molecules in evolving acute and chronic inflammatory models. J Cell Biochem 1993, 17A:353
69. Pelletier RP, Ohye RG, Vanbuskirk A, Sedmak DD, Kincade P, Ferguson RM, Orosz CG: Importance of endothelial VCAM-1 for inflammatory leukocytic infiltration *in vivo*. J Immunol 1992, 149:2473–2481
70. Butcher EC, Scollay RG, Weissman IL: Lymphocyte adherence to high endothelial venules: characterization of a modified *in vitro* assay, and examination of the binding of syngeneic and allogeneic lymphocyte populations. J Immunol 1979, 123:1996–2003
71. Mintz M, Rapaport R, Oleske JM: Elevated serum levels of tumor necrosis factors are associated with progressive encephalopathy in children with acquired immunodeficiency syndrome. Arch Dis Child 1989, 143: 771–774
72. Wahl SM, Allen JB, McCartney-Francis N, Morganti-Kossmann MC, Kossmann T, Ellingsworth L, Mai UEH, Mergenhagen SE, Orenstein JM: Macrophage- and astrocyte-derived transforming growth factor β as a mediator of central nervous system dysfunction in acquired immune deficiency syndrome. J Exp Med 1991, 173:981–991
73. Heyes MP, Brew BJ, Martin A, Price RW, Salazar AM, Sidtis JJ, Yergey JA, Mouradian MM, Sadler AE, Keilp J: Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: relationship to clinical and neurological status. Ann Neurol 1991, 29:202–209
74. van Kooyk Y, van de Wiel-van Kemenade E, Weder P, Huijbens RJF, Figdor CG: Lymphocyte function-associated antigen 1 dominates very late antigen 4 in binding of activated T cells to endothelium. J Exp Med 1993, 177:185–190
75. Cybulsky MI, Fries JWU, Williams AJ, Sultan P, Davis VM, Gimbrone MA, Jr, Collins T: Alternative splicing of human VCAM-1 in activated vascular endothelium. Am J Pathol 1991, 138:815–820
76. Bevilacqua MP, Pober JS, Wheeler ME, Cotran RS, Gimbrone MA, Jr: Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. J Clin Invest 1985, 76:2003–2011
77. Masinovsky B, Urdal D, Gallatin WM: IL-4 acts synergistically with IL-1β to promote lymphocyte adhesion to microvascular endothelium by induction of vascular cell adhesion molecule-1. J Immunol 1990, 145:2886–2895
78. Fischer A, Seger R, Durandy A, Grospierre B, Virelizier JL, Le Deist F, Griscelli C, Fischer E, Kazatchkine M, Bohler M-C, Descamps-Latscha B, Trung PH, Springer TA, Olive D, Mawas C: Deficiency of the adhesive protein complex lymphocyte function antigen 1, complement receptor type 3, glycoprotein p150,95 in a girl with recurrent bacterial infections: effects on phagocytic cells and lymphocyte functions. J Clin Invest 1985, 76:2385–2392
79. Fischer A, Blanche S, Veber F, Delaage M, Mawas C, Griscelli C, Le Deist F, Lopez M, Olive D, Janossy G: Prevention of graft failure by an anti-HLFA-1 monoclonal antibody in HLA-mismatched bone-marrow transplantation. Lancet 1986, II:1058–1061
80. Hildreth JEK, Gotch FM, Hildreth PDK, McMichael AJ: A human lymphocyte-associated antigen involved in cell-mediated lympholysis. Eur J Immunol 1983, 13: 202–208
81. Sánchez-Madrid F, De Landázuri MO, Morago G, Cebrián M, Acevedo A, Bernabeu C: VLA-3: a novel polypeptide association within the VLA molecular complex: cell distribution and biochemical characterization. Eur J Immunol 1986, 16:1343–1349
82. Campanero MR, Pulido R, Ursa MA, Rodríguez-Moya M, De Landázuri MO, Sánchez-Madrid F: An alternative leukocyte homotypic adhesion mechanism, LFA-1/ICAM-1-independent, triggered through the human VLA-4 integrin. J Cell Biol 1990, 110:2157–2165