Endothelial Cell Integrin Laminin Receptor Expression in Multiple Sclerosis Lesions

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Laminin, a major glycoprotein component of vessel basement membranes, is recognized by β_1 - and β_3 -integrins expressed on endothelial cells. To determine how endothelial cell integrins might function in multiple sclerosis (MS) lesions, integrin laminin receptors and laminin were analyzed in central nervous system samples from MS patients and controls by immunohistochemistry. In active MS lesions, endothelial cell VLA-6 and β_1 subunits were decreased compared to controls whereas α_v subunit and VLA-1 were increased. In chronic inactive lesions β_1 , VLA-6 and α_v were the same as controls but VLA-1 remained increased. α_3 subunit was constant in all samples. By immunoelectron microscopy VLA-1, VLA-6, β_1 , and laminin were distributed throughout endothelial cells; α_v was adjacent to and on luminal surfaces; α_v and VLA-1 were on intercellular junctions. These results indicate distinct regulation and functions of these integrins in different lesion stages. In active lesions decreased endothelial cell β_1 /VLA-6 could result in their detachment from laminin thereby facilitating leukocyte transvascular migration and blood-brain barrier breakdown. α_v and VLA-1 on intercellular junctions may participate in re-establishing vessel integrity after leukocyte migration. Luminal surface α_v also likely binds intraluminal ligands and cells. In chronic inactive plaques persistently elevated endothelial cell VLA-1 correlates with long-standing endothelial cell and blood-brain barrier dysfunction. (Am J Pathol 1998, 153:405–415)

Laminins (Ln) are major glycoprotein components of extracellular matrix and vessel basement membranes (BM).¹ Peptide sequences of Ln and of other extracellular matrix molecules are recognized by $\alpha_1\beta_1$ (VLA-1), $\alpha_2\beta_1$ (VLA-2), $\alpha_3\beta_1$ (VLA-3), $\alpha_6\beta_1$ (VLA-6), $\alpha_6\beta_4$, and $\alpha_v\beta_3$ integrins expressed on the surfaces of many cell types.^{2,3} Integrin-mediated recognition of extracellular matrix molecules results in intracellular signaling that affects a range of cell behaviors.⁴ In endothelial cells these signals affect focal adhesions and cytoskeletal organization, ie,

actin fiber assembly. Therefore, integrin-mediated endothelial cell recognition of Ln and other BM molecules may determine cell-to-cell adhesiveness and mediate behaviors such as spreading, retraction, polarization, and migration that are essential for the maintenance and normal functioning of blood vessels.^{5–7}

Inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor- α (TNF- α), interferon- γ (IFN γ), and transforming growth factor- β (TGF β), growth factors such as fibroblast growth factor, and reactive oxygen intermediates induce changes in the levels and surface distribution of endothelial cell integrins *in vitro*.^{8–13} In immune reactions these alterations likely affect endothelial cell recognition of BM molecules and result in contraction of the endothelial cells, producing defects or denudation of the vascular lining. Cytokines may also be bound by Ln¹⁴ and influence extracellular matrix turnover.^{15,16} Thus, both endothelial cell integrin expression and the BM may undergo numerous modifications over the course of cellular immune reactions. Indeed, in diverse inflammatory conditions alterations of endothelial cell integrin Ln receptor expression have been documented.^{17–20} These studies suggest that endothelial cell integrin expression changes over time in complex patterns *in vivo* and that alterations may be specific for each integrin and vascular bed affected.

In central nervous system (CNS) immune reactions, particularly in acute lesions of multiple sclerosis (MS), the blood-brain barrier breaks down as the endothelial cell layer becomes porous and leukocytes migrate across blood vessel walls.^{21,22} Previous studies of MS have demonstrated deposition of plasma and extracellular matrix molecules on endothelial cells in acute and chronic active lesions and modulation of the expression of their integrin receptors.^{23,24} Increased expression of matrix metalloproteinases, enzymes that mediate vascular basement membrane and CNS extracellular matrix turnover, are also found in active MS lesions.^{25,26} Furthermore, in chronic MS plaques blood-brain barrier defects and endothelial cell abnormalities persist and may contribute to a parenchymal extracellular matrix that does not promote or actively impedes tissue repair.^{27–29} Thus, alterations of both endothelial cell integrins and vascular

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Table 1. Case Material

Diagnoses	No. of cases	No. of samples studied
Demyelinating diseases		
Acute MS*	3	20
Chronic MS†	10	25
Acute disseminated leucoencephalitis‡	1	1
Other neurological diseases		
Adrenoleukodystrophy§	2	2
Acute herpes simplex encephalitis¶	2	2
Cerebral infarcts	3	4
Hypoxic-ischemic encephalopathy	3	4
Cervical cord compression**	1	1
Normal CNS††	20	23

*One biopsy sample from a 37-year-old woman, disease duration not known; one biopsy sample from a 35-year-old woman, disease duration 6 weeks; one autopsy sample from a 30-year-old man, disease duration 11 months.

†There were 6 women and 4 men in cases for which information is available; mean age = 51.3 years (range, 33 to 78 years); clinical disease duration mean = 14.4 years (range, 3 to 37 years); postmortem intervals mean = 9.1 hours (range, 2 to 23 hours).

‡Biopsy sample from a 52-year-old man, clinical disease duration 3 to 4 weeks.

§22- and 10-year-old males. Samples contain noninflammatory chronic lesions.

¶One cerebral cortical biopsy sample and one autopsy sample.

||Infarct ages were approximately 2 days, 4 to 5 days, and remote.

**Secondary to vertebral metastases of adenocarcinoma.

††Neuropathologically normal autopsy samples were from cerebral hemispheres (*n* = 12), brain stem (*n* = 2), cerebellum (*n* = 3), optic nerve (*n* = 1), and spinal cord (*n* = 5). In cases for which information was available, there were 7 women and 11 men; mean age = 58.6 years (range, 5 days to 85 years); Mean postmortem interval = 11.7 hours (range, 3 to 22 hours).

BM components are implicated in the pathogenesis of all stages of MS lesions.

To delineate more precisely the molecular interactions between endothelial cells and Ln that occur in MS, vascular endothelial cell integrin Ln receptors and Ln in MS lesions and controls were analyzed by immunohistochemistry. Immunoelectron microscopy was used to characterize subcellular localizations of individual integrins that indicate their potential functions *in situ*. The results indicate complex expression patterns of endothelial cell integrin Ln receptors in different MS lesion stages and provide insight into molecular mechanisms of endothelial cell pathobiology in CNS inflammatory conditions and of blood-brain barrier dysfunction in chronic MS plaques.

Materials and Methods

Case Material (Table 1)

Samples of CNS tissues were obtained from cerebral hemisphere biopsies and from autopsies at Massachusetts General Hospital (Boston, MA), Stanford University Medical Center (Stanford, CA), and Veterans Affairs Health Care System (Palo Alto, CA). Additional autopsy samples from patients with MS were obtained from the Brigham and Women's Hospital (Boston, MA), the National Neurological Research Bank, VA Wadsworth Medical Center (Los Angeles, CA), and the Rocky Mountain

MS Tissue Bank (Englewood, CO). The samples were frozen and stored at -80°C in OCT compound (Miles Laboratories, Naperville, IL).

MS Lesion Classification

Lesions in MS tissue samples were determined to be "active" by the presence of perivascular mononuclear cell infiltrates and by the detection of numerous Oil Red O-positive myelin breakdown products in macrophages in adjacent serial sections. In autopsy samples, particularly from patients in whom the disease had been present for many years, the precise ages of specific lesions are not known and even in acute MS patients whose clinical disease courses were very brief (ie, less than one year), lesions with these features were the most prevalent. Therefore, lesions classified as "active" may have included very acute and remyelinating lesions, but the vast majority were more likely chronic active lesions or plaques.³⁰ Lesions without myelin, evidence of ongoing myelin degeneration (ie, Oil Red O-positive macrophages), or recognizable mononuclear cell infiltrates were designated as inactive, demyelinated lesions ("chronic inactive"). These were the most numerous lesions in the chronic MS autopsy samples. Areas in samples from MS patients with intact white matter, as judged by tissue density on gross inspection and microscopic confirmation of the presence of intact myelinated axons, were classified as MS normal-appearing white matter (NAWM). Using these criteria, these lesions/compartments were uniform in comparisons among the different MS cases.

Monoclonal Antibodies and Immunohistochemistry

Cryostat sections of 6- μm thickness were stained with the monoclonal antibodies (mAb) listed in Table 2 diluted in phosphate-buffered saline, pH 7.4 (PBS), using immunoperoxidase as described.²⁴ In brief, air-dried sections were fixed in acetone, washed in PBS, and incubated sequentially in 10% normal horse serum, mAb, 0.03% H_2O_2 in PBS, biotinylated horse anti-mouse immunoglobulin (Ig) (Vector Laboratories, Burlingame, CA), and avidin-biotin-horseradish peroxidase complex (Vector), with washes in PBS between incubation steps. Immunoperoxidase reaction product was visualized with 3-amino-9-ethyl carbazole (Aldrich Chemical Co., Milwaukee, WI) and fixed in formol acetate. The sections were counterstained with hematoxylin. Samples of normal spleen were used as positive controls and for determinations of optimal staining dilutions for each mAb. Negative staining controls included substitution of irrelevant mAb and PBS for primary antibodies. Staining of normal and pathological tissues was done concurrently and there were uniform exposures to each reagent.

For semiquantitative analysis of stained cryosections, stained vessels in CNS white matter were counted by two independent observers and areas in which counts were done were calculated from ruler measurements. Vessels seen in cross-section with any endothelial cell staining were counted as positive. In samples from MS patients,

Table 2. Monoclonal Antibodies

Antigen	Clone	Isotype	Source	Staining dilution*	Reference No.
CD49a (VLA-1, $\alpha_1\beta_1$)	TS2/7	Mouse IgG ₁	T Cell Diagnostics (Cambridge, MA)	1:100	31
CD49b (VLA-2, α_2 subunit)	Gi9	Mouse IgG ₁	Life Technologies (Rockville, MD)	1:500	32
CD49c (VLA-3, α_3 subunit)	P1B5	Mouse IgG ₁	Life Technologies	1:1000	33
CD49f (VLA-6, α_6 subunit)	GoH3	Rat IgG _{2a}	AMAC (Westbrook, ME)	1:100	34
CD51 (α_v subunit)	VNR147	Mouse IgG ₁	Life Technologies	1:500	35
CD29 (β_1 subunit)	P4C10	Mouse IgG ₁	Life Technologies	1:500	36
CD61 (β_3 subunit, gpIIla)	SZ.21	Mouse IgG ₁	AMAC	1:500	37
Human laminin	LAM-1	Rat IgG _{2b}	BioGenex Laboratories (San Ramon, CA)	1:100	38

*Optimal staining dilution for light microscopy.

separate counts were made in regions classified as active lesions, chronic inactive lesions, and NAWM. Stained vessels in normal white matter and white matter lesions were counted in the other cases. Sample preservation and the presence of vessel staining determined which areas were selected for counting. Student's *t*-test was used for statistical analysis.

Immunoelectron Microscopy and Morphometric Analysis

Tissues from OCT blocks containing normal optic nerve, other normal CNS samples, and active MS lesions were thawed, rinsed in 10 mmol/L PBS with 2 mmol/L NaN₃ (PBSem) for 90 minutes with three changes to remove the OCT. Samples (500 μ m thick) were sliced and the rinsed tissue was placed in 3.0% paraformaldehyde/0.2% glutaraldehyde in PBSem and fixed for 3 hours at room temperature (RT) with mixing. The fixed tissues were then rinsed in PBSem, cut into smaller blocks, and stored overnight at 2–8°C. The tissue blocks were then dehydrated for 10 minutes each in 50% and 70% ethanol and for 10 minutes each three times with 96% ethanol. The blocks were immediately infused with 100% LR-White (Sigma, St. Louis, MO) for 60 minutes at RT, followed by 100% LR-White overnight at 0–4°C. and 100% LR-White for 60 minutes at RT. The infusion steps were all performed in the dark with rotary agitation. For polymerization the samples were transferred to size 1 gelatin capsules and incubated at 50 $\pm$ 1°C. for 40 hours. Ultrathin (80- to 90-nm) sections were cut from these samples using a microtome (Reichert Om U2, Leica Inc., Deerfield, IL) and were transferred to formvar-covered carbon-coated nickel grids.

Immunostaining was carried out at RT unless otherwise noted. The grids were incubated in 20 mmol/L glycine in 10 mmol/L PBS for 10 minutes at 37°C, blocked with 5% normal goat serum in 10 mmol/L PBS with 0.8% BSA and 0.1% IGSS gelatin (PBSAG) for 30 minutes, mAb diluted 1:50 in PBSAG with 1% normal goat serum for 2 hours at RT and then overnight at 2–4°C. The optimal dilution for each mAb was determined in preliminary studies to max-

imize labeling and eliminate nonspecific staining of cells, nuclei, and BMs. The grids were then incubated with the appropriate 15-nm gold particle-conjugated goat anti-mouse or anti-rat IgG reagent (E-Y Laboratories, Inc., San Mateo, CA) diluted 1:25 in PBSAG with 1% normal goat serum for 2 hours at RT. PBS washes were performed between each step and after the gold-labeled antibody incubation. The grids were then postfixed in 2% glutaraldehyde in PBSem for 10 minutes, washed in PBS followed by deionized water (DIH₂O). They were then stained with 4% uranyl acetate oxalate, washed with boiled DIH₂O, washed with 20 mmol/L NaOH, and further stained with 0.2% lead citrate in a Petri dish with NaOH granules. The grids were then washed once in NaOH and in boiled DIH₂O four times, air-dried, and examined in a Zeiss electron microscope. Fields for photographs were selected on the basis of tissue preservation and the presence of multiple gold particles. Immunoelectron microscopy was performed on samples for VLA-1, VLA-6, α_v and β_1 subunits, and Ln. A spleen sample was used as a positive control and normal mouse serum substituted for mAb as a negative control.

Localizations of the gold particles within endothelial cell cytoplasm were assessed in electron photomicrographs of preparations stained for VLA-6 and α_v and β_1 subunits. Distances of each particle in the cytoplasm from the luminal surface (D_{Lum}) and from the abluminal surface (D_{Ablum}) were measured and a D_{Lum}/D_{Ablum} ratio was determined for each particle. A ratio of <1 indicates that the particle is localized closer to the luminal surface and >1 indicates that the particle is closer to the abluminal surface.

Results

VLA-6 and β_1 Subunit

VLA-6 was abundantly expressed in a uniform pattern on capillaries and small venules in normal white matter (Figure 1A). Endothelial cells were also stained in gray matter, leptomeninges, and spinal nerve roots. Vascular smooth muscle, neurons, and glia were VLA-6-negative.

Figure 1. Immunolocalization of VLA-6. All are mAb GoH3 with hematoxylin. **A:** Normal control white matter. Numerous capillaries express VLA-6. Magnification, $\times 114$. **B:** Active MS lesion. Endothelial cells in approximately the lower third of this vessel are VLA-6-positive whereas there is minimal staining of most of the vessel. Perivascular mononuclear inflammatory cells and other cells in the parenchyma are VLA-6-negative. Magnification, $\times 571$. **C:** Chronic inactive MS plaque. Many vessels express VLA-6. Magnification, $\times 114$.

In MS NAWM, compared to normal white matter, VLA-6 vessel staining was slightly decreased. Many vessels with perivascular inflammatory cells in active lesions were either completely negative or had incomplete staining, ie, only a few stained endothelial cells (Figure 1B). Inflammatory cells and foamy macrophages were mostly VLA-6-negative. In chronic inactive MS plaques the extent of vessel staining was similar to that in normal white matter (Figure 1C). In other inflammatory and noninflammatory conditions VLA-6 expression was also observed but no consistent patterns of immunostaining were identified and counts of stained vessels were not done in those samples.

Microvascular Expression of VLA-6 and Integrin Beta-1 Subunit

Figure 2. Numbers of VLA-6-positive vessels/mm² are less in MS NAWM (samples from 5 cases) and active MS lesions (samples from 3 cases) compared to normal controls (samples from 14 cases). In chronic inactive lesions (samples from 6 cases) the numbers of stained vessels/mm² are the same as in controls. Similar patterns are observed for the β_1 -integrin subunit. Data are from a single observer but staining counts and patterns were confirmed by two additional observers. Numbers of sample fields counted in each group are indicated beneath each bar. Asterisks indicate significant differences compared to normal controls, as described in Results. Error bar = SEM.

As previously reported and illustrated,²⁴ expression of the β_1 -integrin subunit was prominent on CNS microvessels in control white matter and was reduced in active MS lesions compared to control and MS NAWM. Staining patterns were similar to those of VLA-6 but in active lesions additional staining of macrophages and other cells was also noted.

Results of semiquantitative analysis of vascular staining for VLA-6 and β_1 subunit are shown in Figure 2. Numbers of VLA-6-positive vessels/mm² were lower in MS NAWM compared to control white matter ($P < 0.01$) and in active MS plaques ($P < 0.02$ compared to MS NAWM, $P < 0.001$ compared to control white matter). Similarly, active MS plaques had fewer β_1 subunit-positive vessels than were found in MS NAWM ($P < 0.02$) or controls ($P < 0.01$). Numbers of VLA-6- and β_1 subunit-positive vessels/mm² were the same in chronic inactive MS lesions as in controls.²⁴

By immunoelectron microscopy in normal CNS samples immunogold labeling for VLA-6 subunit was found to be dispersed throughout endothelial cell cytoplasm. Although no striking polarization of labeling was identified many gold particles were found on or near abluminal cell surfaces (Figure 3). In active lesions there were fewer gold particles on endothelial cells and other cells were negative. Immunogold labeling for β_1 subunit was also dispersed through endothelial cell cytoplasm in normal samples. As expected from light microscopic analyses and previous studies, cells in perivascular and parenchymal areas in active MS lesions were also labeled for the β_1 subunit (not shown).

VLA-1 and α_v Subunit

VLA-1 expression was observed on small numbers of microvessels in control and MS NAWM (Figure 4A). Vessel expression was increased in active MS lesions and

Figure 3. Immunoelectron microscopic localization of VLA-6 in endothelial cell cytoplasm. Numerous immunogold particles labeling VLA-6 are distributed throughout the endothelial cell cytoplasm in a microvessel endothelial cell from a normal control optic nerve sample. Several gold particles are located on the abluminal surface adjacent to the BM. Absence of staining on the BM indicates specificity of the gold particle labeling. L, lumen. mAb GoH3. Magnification, $\times 20,300$

appeared to be localized on endothelial cells. No VLA-1 expression on mononuclear or CNS resident cells was observed (Figure 4B). Vessel expression of VLA-1 was as prominent in chronic inactive MS lesions as in active MS lesions (Figure 4C). Small numbers of VLA-1-positive vessels were also observed in other neurological disease controls with no clear trends identified.

In sections stained for the α_v subunit, endothelial cell localization, vessel staining, and an increase in expression in active MS lesions similar to that of VLA-1 were observed. The α_v subunit was additionally expressed on macrophages and astrocytes in active lesions (Figure 4D).

Results of semiquantitative analysis of VLA-1 and α_v subunit are shown in Figure 5. In active MS lesions numbers of VLA-1-positive vessels/mm² were higher than in controls and MS NAWM ($P < 0.01$ for both). In chronic inactive MS lesions numbers of VLA-positive vessels/mm² remained significantly greater than in control and MS NAWM ($P < 0.03$ for combined data). Similar numbers of stained vessels/mm² for α_v were seen in active MS lesions, but in contrast to VLA-1, α_v -positive vessels/mm² were the same as in controls in chronic inactive lesions.²⁴

By immunoelectron microscopy detection of VLA-1 was generally low but when gold particles were found in endothelial cell cytoplasm, they appeared to be randomly distributed. Specific labeling for VLA-1 was present, however, on interendothelial cell junctions and adjacent areas (Figure 6). No VLA-1 was observed on parenchymal or other cells or on the BM.

In control samples, α_v subunit immunogold labeling was extremely sparse, but in samples with active MS lesions endothelial cell labeling was more abundant. Individual and clustered gold particles were localized on or near endothelial cell membrane luminal surfaces. Within the cytoplasm they were found more frequently in the luminal than abluminal portions (Figure 7). Labeling was also specifically localized on or near intercellular tight

junctions (Figures 7 and 8). Some labeled cell fragments within the vessel lumens may represent platelets. Scattered particles were also seen on perivascular and parenchymal cells.

Morphometric analyses demonstrated that in each of seven endothelial cells labeled for α_v subunit, more gold particles were localized within the cytoplasm closer to the luminal than the abluminal surface (Figure 9A). By contrast, in a similar analysis of endothelial cells immunolabeled for VLA-6, no consistent intracytoplasmic polarization of gold particle localization was evident, indicating a random distribution (Figure 9B).

α_3 and α_2 Subunits

In control samples meningeal artery smooth muscle and rare parenchymal venules were α_3 subunit-positive whereas gray and white matter were otherwise negative. In active MS lesions small numbers of white matter microvessels were stained but there was no clear relationship between the presence of perivascular inflammatory cells and α_3 vascular expression (Figure 10A). In some active plaques more diffuse staining of macrophages and glia, including Creutzfeldt astrocytes,²² were observed (not shown). Similar patterns of staining of α_3 -positive vessels were seen in inactive MS lesions and no differences in the numbers of vessels stained in the different sample groups were identified (Figure 5).

The anti- α_2 subunit mAb immunostained neuron cell bodies in gray matter but no consistent vessel staining in normal or pathological tissues was observed. Some active MS samples showed prominent staining of macrophages and astrocytes and old plaques showed high diffuse background staining (not shown).

Ln

By light microscopy Ln was detected on large meningeal vessels and on arteries, venules, and microvessels in

Figure 4. VLA-1 and α_v subunit immunostaining. A to C, mAb TS2/7 with hematoxylin. A: Two capillaries (arrows) express VLA-1 whereas a larger vessel (right center) is mostly VLA-negative in normal control white matter. Magnification, $\times 114$. B: Prominent staining of a venule in an active MS lesion. Perivascular mononuclear cells, astrocytes (arrowheads) and other cells are unstained. Magnification, $\times 171$. C: Numerous capillaries retain immunoreactivity in a chronic inactive MS plaque. Magnification, $\times 114$. D: α_v subunit is expressed on a venule and on parenchymal macrophages and astrocytes in an active MS lesion. mAb VNR 147. Magnification, $\times 171$.

Figure 5. VLA-1- and α_v subunit positive microvessels/mm² are more numerous in active MS lesions (samples from 4 cases) than in NAWM (samples from 6 cases) and controls (samples from 14 cases). Numbers of VLA-1-positive microvessels in chronic inactive lesions (samples from 6 cases) are also higher than in control samples whereas numbers of α_v subunit-positive microvessels are the same as controls. α_3 integrin subunit microvessel staining is essentially the same in all samples and conditions. See also legend to Figure 2.

Figure 6. Immunoelectron microscopic localization of VLA-1 on interendothelial cell junction (arrow) in a normal optic nerve microvascular endothelial cell. Three gold particles are on and two additional particles are in the general vicinity of the junction. Background labeling of cytoplasm, lumen and BM are minimal. L, lumen. mAb TS2/7. Magnification, $\times 20,200$.

Figure 7. Immunoelectron microscopic localization of α_6 subunit in microvascular endothelial cells in the thalamus of a patient with acute MS. Many gold particles are on the luminal side of the perinuclear cytoplasm. There are fewer particles on the abluminal portion of cytoplasm and none on the BM. Particles are also clustered on and in the region of intercellular junctions (lower right side of field, higher power in inset). Immunogold labeling with VNRI47 mAb and 15 nm gold particles. L, Lumen. Magnification, $\times 12,700$; inset, $\times 20,000$.

normal CNS parenchyma (Figure 10B). In active lesions vessel staining was sometimes disrupted or not apparent (Figure 10C). These alterations were focal, however, and no significant differences in numbers of stained vessels among the sample groups were observed (data not shown). By immunoelectron microscopy Ln labeling was observed in endothelial cells and predominantly on the BM but not in pericytes or other cells (Figure 11).

Discussion

This study was undertaken to understand how the expression of integrin Ln receptors and their recognition of BM Ln may contribute to the physiological alterations and functions of CNS microvascular endothelial cells over the course of evolution of MS lesions. The necessary use of autopsy tissue samples from MS patients for such analyses is complicated by the fact that specific lesions' ages are unknown and the majority of lesions likely were present for many years.²² Although the use of samples from clinically acute MS cases improves the likelihood that specific lesions are pathologically early, the precise stages of endothelial cell activation and injury in the samples are not known. Thus, some of the present observations, even in active MS lesions, may well relate to more chronically altered or regenerating rather than acutely injured endothelial cells or they may reflect the

simultaneous occurrence of both types of processes. Furthermore, cellular expression of β_1 integrins does not necessarily completely correlate with affinity for specific extracellular matrix molecule ligands⁴¹ and integrins clearly have complex binding capacities and multiple biological roles.^{3,4,42} Therefore, inferences of specific functions based solely on integrin and integrin subunit expression need to be made with caution.

On the other hand, the findings on immunostaining of specific integrins in control tissue samples were essentially as reported in other studies of human CNS tissues^{39,40} and the alterations identified in MS lesions are similar to those reported in non-CNS inflammatory conditions. Furthermore, the immunostaining pattern and subcellular localizations of specific integrins identified are also similar to those in human endothelial cells *in vitro* and many of the alterations identified in the present study can also be induced in these cells by specific inflammatory mediators. Thus, the complex patterns of integrin Ln receptor expression identified are likely relevant to acute as well as chronic MS lesions *in vivo*.

Vascular expression of VLA-6 (α_6 subunit) was less in MS NAWM and in active MS lesions than in normal controls. Down-regulation of the β_1 subunit expression paralleled that of VLA-6, but in view of the multiple potential pairings of the β_1 subunit with other α chains, it is not certain whether these patterns are directly related. In-

Figure 8. Interendothelial cell junction in a microvessel adjacent to the vessel in Figure 7 shows gold particle labeling on the junctional complex (arrow) and on the luminal surface (arrowheads) but not in the abluminal cytoplasm or on the BM. L, Lumen. Magnification, $\times 20,300$.

deed, decreased β_1 expression has been reported in a model of vascular injury⁴³ and may therefore be a more general indicator of endothelial cell injury. Down-regulation of α_6 subunit expression has also previously been documented in inflamed proliferative synovia from patients with rheumatoid arthritis^{17,19} and this effect can be mimicked in synovial cells using a combination of TNF- α and IFN- γ *in vitro*.⁴⁴ Because these cytokines are present

in inflammatory MS lesions,^{45,46} it is likely that the effects on VLA-6 expression in inflammatory MS lesions are similarly mediated at least in part by these cytokines. In addition, certain chemokines may also modulate β_1 integrin-mediated T cell affinity for Ln and other extracellular matrix proteins without altering β_1 expression levels.⁴⁷ These chemokines are also likely present in inflammatory MS lesions⁴⁸ and they may modify endothelial cell proliferation.⁴⁹ It is not known at present, however, if they affect human CNS endothelial cell-extracellular matrix molecule interactions.

The observed patterns of VLA-6 expression generally parallel changes in vessel wall integrity and blood-brain barrier function, ie, both may be severely compromised in acute lesions.²¹ Furthermore, the significant although lesser difference in VLA-6-positive vessel staining in the NAWM samples compared to the controls might correlate with abnormalities in blood-brain barrier function, ie, increased water content, that are detected in MS NAWM in patients in areas where there is little or no histological evidence of injury.⁵⁰ Because VLA-6 may be involved in matrix guidance pathways that permit endothelial cells to locate each other in angiogenesis and other pathological processes,⁶ VLA-6 down-regulation might be related to disengagement of endothelial cells from BM Ln and from each other. Indeed, a recent study suggests that in the absence of engagement with an extracellular matrix molecule substrate VLA-6 may contribute to cell motility.⁵¹ Thus, in active MS lesions VLA-6 down-regulation in endothelial cells would result in their detachment from Ln and this might both facilitate leukocyte passage through the vessel wall and contribute to the characteristic breaches of the blood-brain barrier. The apparent return of VLA-6 and β_1 subunit staining patterns to normal in chronic lesions implies that these aspects of endothelial cell dysfunction may be reversible.

In contrast to VLA-6 and β_1 subunit, vascular α_v subunit was increased in active MS lesions. An increase in α_v expression is consistent with the finding of enhanced α_v

Figure 9. Morphometric analysis of subcellular localizations of α_v -integrin and VLA-6. Data are expressed as numbers of gold particles with D_{lum}/D_{ablum} ratios < 1 or > 1 in each endothelial cell analyzed. **A:** In photomicrographs of samples immunolabeled for α_v integrin subunit, there were more intracytoplasmic gold particles with D_{lum}/D_{ablum} ratios < 1 in each of seven cells, indicating that in each cell more particles were closer to or on the luminal surface than were close to the abluminal surface. $P = 0.0006$ by Fisher test. **B:** In contrast to data in **A**, in ten endothelial cells immunolabeled for VLA-6 the D_{lum}/D_{ablum} ratios did not show a consistent pattern of localization, ie, in seven cells there were more particles closer to abluminal surfaces and in three there were more particles closer to luminal surfaces, indicating random distribution of particles within the cytoplasm.

Figure 10. α_3 subunit and Ln immunostaining. **A:** Active MS plaque with a stained venule and diffuse immunoreactivity for α_3 subunit. mAb P1B5. Magnification, $\times 229$. **B:** Ln in MS NAWM. Several microvessels in the field are stained. mAb LAM1. Magnification, $\times 114$. **C:** Ln in an active MS lesion. BM Ln appears disrupted by migrating inflammatory cells and one vessel (arrow) shows no immunostaining. mAb LAM1. Magnification, $\times 229$.

and $\alpha_v\beta_3$ on microvessels in experimental cerebral ischemic lesions by Okada et al,⁵² although in that study up-regulation was found in smooth muscle rather than endothelial cells. On the other hand, Defilippi et al⁵³ showed that $\alpha_v\beta_3$ on cultured human umbilical vein endothelial cells (HUVECs) decreases when they are treated with TNF- α and IFN- γ and that this is due to a selective effect on the β_3 rather than the α_v subunit. Because these cytokines are present in active MS lesions, the explanation for this possible discrepancy with the present data are unclear but differences between the *in vitro* and *in vivo* conditions, the type of cells studied, and the timing and levels of cytokines are likely signifi-

Figure 11. Immunoelectron microscopic localization of Ln in a thalamic microvessel from a patient with chronic MS. Gold particles are present in endothelial cell cytoplasm (e) and on the BM (arrowheads) but to a lesser extent on the cytoplasm of pericytes (p). **Inset** shows particles on BM from the left side of field. Immunogold labeling with mAb LAM-1. L, Lumen. Magnification, $\times 11,300$; **inset**, $\times 20,000$.

cant. In contrast, our findings of luminal surface expression and intracytoplasmic localization of α_v in CNS endothelial cells are consistent with the polarization of $\alpha_v\beta_3$ demonstrated in HUVECs by Conforti et al.⁵⁴ Luminal surface expression implies the potential for α_v -integrins to bind to plasma molecules, including fibronectin, fibrinogen and vitronectin, and to intraluminal leukocytes that have these RGD-containing proteins bound on their surfaces.⁵⁵ The membrane localization also implies that endothelial cell α_v or portions of it may be shed into the circulation to a greater degree than would molecules bound to the BM. Therefore, their detection in MS patients might indicate disease activity. Up-regulation of α_v expression also correlates with other evidence of endothelial cell activation in MS lesions (Van der Maesen et al, manuscript in preparation).^{56–58} As in HUVECs,⁵⁹ the α_v subunit was also found on interendothelial cell junctions. This localization correlates with the dynamic redistribution of these molecules and cytoskeletal reorganization induced in HUVECs by H₂O₂ and TNF *in vitro*^{11,12} and likely also relates to their loss of intercellular adhesiveness.

In active lesions microvascular endothelial cell immunostaining for VLA-1 was also greater than that in normal samples. Unlike α_v subunit, however, VLA-1 was not preferentially localized to luminal membranes and the amount of immunostaining was also greater in chronic lesions than in controls. Therefore, VLA-1 is differently regulated and likely has functions distinct from α_v -integrins. Indeed, VLA-1 expression is increased in HUVECs by TNF- α and other inflammatory mediators and this enhanced expression is associated with increased adhesiveness to Ln as

well as to other BM components.⁶⁰ Increased endothelial cell adhesion to the BM would presumably be more important as the endothelial cell lining becomes re-established after leukocytes have migrated through the vessel wall than during active inflammation. The localization of VLA-1 on interendothelial junctions supports this view because it implies involvement in maintaining tight intercellular contacts. The absence of a detectable increase in the amount of VLA-1 in NAWM (Figure 5) is also consistent with a role for VLA-1 in later stages because endothelial cells in areas where the white matter is intact, even if they are functionally abnormal, would not have undergone the same degree of pathological alteration as those in acute and chronic lesions. Although endothelial cells in chronic MS plaques show evidence of impaired barrier function, specific morphological abnormalities of interendothelial cell tight junctions have not been identified to date.²⁸ Nevertheless, the failure of VLA-1 to return to normal patterns of expression in chronic lesions might indicate that at the molecular level the junctions continue to be abnormal, and this could be related to persistent blood-brain barrier dysfunction of chronic plaques.²⁷

As documented in previous studies of MS lesions,⁶¹ Ln immunoreactivity was primarily localized around blood vessels and there was slight BM Ln thickening in chronic plaques. Ln immunoreactivity may be fixation-dependent,⁶² and there are also newly discovered Ln variants in the CNS.⁶³ Therefore, a more comprehensive survey of the various Ln chains and molecular isoforms might demonstrate additional alterations in Ln and other BM component immunoreactivity in MS lesions. In some active lesions vascular Ln did appear to be disrupted and even to disappear from vessels (Figure 10C), suggesting that there may be active breakdown of BM Ln in conjunction with the inflammation. Indeed, a cytokine-dependent endothelial cell-derived sulfatase that can degrade subendothelial BM has recently been described⁶⁴ and proteolytic digestion of Ln may release peptides with immunological functions not present in intact Ln.⁶⁵ Thus, endothelial cells actively modify as well as respond to their extracellular matrix environment as part of the immunopathological response.

In conclusion, we have identified alterations in the expression and subcellular localization of endothelial cell integrin Ln receptors in MS lesions. In active lesions decreased β_1 /VLA-6 may result in endothelial cell detachment from BM, thereby facilitating leukocyte emigration and blood-brain barrier breakdown. Enhanced α_v subunit could promote endothelial cell binding to other ligands and cells as well as to Ln and, because of its luminal surface localization, may be shed into the circulation. The α_v subunit and VLA-1 may be particularly important in re-establishing vessel wall integrity and the blood-brain barrier through intercellular junctions. These data support many *in vitro* observations on endothelial cell pathophysiology in immune reactions and provide new insights into mechanisms of endothelial cell dysfunction over the course of evolution of MS lesions. The findings may also have implications for the diagnosis of active disease and for therapeutic targeting of specific endothelial cell molecules in MS patients.

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References

- Paulsson M: Basement membrane proteins: structure, assembly, and cellular interactions. *Crit Rev Biochem Mol Biol* 1992, 27:93-127
- Chan BM, Kassner PD, Schiro JA, Byers HR, Kupper TS, Hemler ME: Distinct cellular functions mediated by different VLA integrin α subunit cytoplasmic domains. *Cell* 1992, 68:1051-1060
- Sonnenberg A: Integrins and their ligands. *Curr Top Microbiol Immunol* 1993, 184:7-35
- Clark EA, Brugge JS: Integrins and signal transduction pathways: the road taken. *Science* 1995, 268:233-239
- Dejana E, Raiteri M, Resnati M, Lampugnani MG: Endothelial integrins and their role in maintaining the integrity of the vessel wall. *Kidney Int* 1993, 43:61-65
- Davis GE, Camarillo CW: Regulation of endothelial cell morphogenesis by integrins, mechanical forces, and matrix guidance pathways. *Exp Cell Res* 1995, 216:113-123
- Shattil SJ, Ginsberg MH: Perspective series: integrin signaling in vascular biology. *J Clin Invest* 1997, 100:1-5
- Basson CT, Kocher O, Basson MC, Asis A, Madri JA: Differential modulation of vascular cell integrin and extracellular matrix expression *in vitro* by TGF β 1 correlates with reciprocal effects on cell migration. *J Cell Physiol* 1992, 153:118-128
- Defilippi P, Silengo L, Tarone G: Regulation of adhesion receptors expression in endothelial cells. *Curr Top Microbiol Immunol* 1993, 184:87-98
- Sepp NT, Cornelius LA, Romani N, Li LJ, Caughman SW, Lawley TJ, Swerlick RA: Polarized expression and basic fibroblast growth factor-induced down-regulation of the $\alpha 6 \beta 4$ integrin complex on human microvascular endothelial cells. *J Invest Dermatol* 1995, 104:266-279
- Bradley JR, Thiru S, Pober JS: Hydrogen peroxide-induced endothelial retraction is accompanied by a loss of the normal spatial organization of endothelial cell adhesion molecules. *Am J Pathol* 1995, 147:627-641
- Bradley JR, Pober JS: Prolonged cytokine exposure causes a dynamic redistribution of endothelial cell adhesion molecules to intercellular junctions. *Lab Invest* 1996, 75:463-472
- Petzelbauer E, Springhorn JP, Tucker AM, Madri JA: Role of plasminogen activator inhibitor in the reciprocal regulation of bovine aortic endothelial and smooth muscle cell migration by TGF- β 1. *Am J Pathol* 1996, 149:923-931
- Hershkoviz R, Goldkorn I, Lider O: Tumour necrosis factor- α interacts with laminin and functions as a pro-adhesive cytokine. *Immunology* 1995, 85:125-130
- Partridge CA, Horvath CJ, Del Vecchio PJ, Phillips PG, Malik AB: Influence of extracellular matrix in tumor necrosis factor-induced increase in endothelial permeability. *Am J Physiol* 1992, 263:L627-L633
- Wyss-Coray T, Feng L, Masliah E, Ruppe MD, Lee HS, Toggas SM, Rockenstein EM, Mucke L: Increased central nervous system production of extracellular matrix components and development of hydrocephalus in transgenic mice overexpressing transforming growth factor- β 1. *Am J Pathol* 1995, 147:53-67
- Nikkari L, Aho H, Yli-Jama T, Larjava H, Jalkanen M, Heino J: Expression of integrin family of cell adhesion receptors in rheumatoid synovium- $\alpha 6$ integrin subunit in normal and hyperplastic synovial lining cell layer. *Am J Pathol* 1993, 142:1019-1027
- Kagami S, Border WA, Ruoslahti E, Noble NA: Coordinated expression of β 1 integrins and transforming growth factor- β -induced matrix proteins in glomerulonephritis. *Lab Invest* 1993, 69:68-76
- Johnson BA, Haines GK, Harlow LA, Koch AE: Adhesion molecule expression in human synovial tissue. *Arthritis Rheum* 1993, 36:137-146

20. Creamer D, Allen M, Sousa A, Poston R, Barker J: Altered vascular endothelium integrin expression in psoriasis. *Am J Pathol* 1995, 147: 1661–1667
21. Gay D, Esiri M: Blood-brain barrier damage in acute multiple sclerosis plaques—an immunocytochemical study. *Brain* 1991, 114:557–572
22. Sobel RA: The pathology of multiple sclerosis. Symposium on Multiple Sclerosis. Edited by Antel J. *Neurol Clin North America*, 1995, 13: 1–21
23. Sobel RA, Mitchell ME: Fibronectin in multiple sclerosis lesions. *Am J Pathol* 1989, 135:161–169
24. Sobel RA, Chen M, Maeda A, Hinojosa JR: Vitronectin and integrin vitronectin receptor localization in multiple sclerosis lesions. *J Neuropathol Exp Neurol* 1995, 54:202–213
25. Maeda A, Sobel RA: Matrix metalloproteinases in the normal human central nervous system, microglial nodules and multiple sclerosis lesions. *J Neuropathol Exp Neurol* 1996, 55:300–309
26. Cossins JA, Clements JM, Ford J, Miller KM, Pigott R, Vos W, Van Der Valk P, De Groot CJDA: Enhanced expression of MMP-7 and MMP-9 in demyelinating multiple sclerosis lesions. *Acta Neuropathol* 1997, 94:590–598
27. Kwon EE, Prineas JW: Blood-brain barrier abnormalities in longstanding multiple sclerosis lesions: an immunohistochemical study. *J Neuropathol Exp Neurol* 1994, 53:625–636
28. Claudio L, Raine CS, Brosnan CF: Evidence of persistent blood-brain barrier abnormalities in chronic-progressive multiple sclerosis. *Acta Neuropathol* 1995, 90:228–238
29. Sobel RA: The extracellular matrix in multiple sclerosis lesions. *J Neuropathol Exp Neurol* 1998, 57:205–217
30. Prineas JW, McDonald WI: Demyelinating diseases. Greenfield's Neuropathology, sixth edition, Graham I, Lantos PL, eds. Oxford University Press, New York, 1997, pp 813–896
31. Hemler ME, Jacobson JG, Strominger JL: Biochemical characterization of VLA-1 and VLA-2: cell surface heterodimers on activated T cells. *J Biol Chem* 1985, 260:15246–15252
32. Santoso S, Kiefel V, Müller-Eckhardt C: Immunochemical characterization of the new platelet alloantigen system Br^a/Br^b. *Br J Haematol* 1989, 72:191–198
33. Wayner EA, Carter WG, Piotrowicz RS, Kunicki TJ: The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. *J Cell Biol* 1988, 107: 1881–1891
34. Sonnenberg A, Janssen H, Hogervorst F, Calafat J, Hilgers J: A complex of platelet glycoproteins Ic and IIa identified by a rat monoclonal antibody. *J Biol Chem* 1987, 262:10376–10383
35. Freed E, Gailit J, van der Geer P, Ruoslahti E, Hunter T: A novel integrin β subunit is associated with the vitronectin receptor α subunit (α_v) in a human osteosarcoma cell line and is a substrate for protein kinase C. *EMBO J* 1989, 8:2955–2965
36. Wayner EA, Orlando RA, Cheresh DA: Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ contribute to cell attachment to vitronectin but differentially distribute on the cell surface. *J Cell Biol* 1995, 113:919–929
37. Ruan C, Du X, Wan H, Hu, X, Li P: Characterization of the fibrinogen binding sites using monoclonal antibodies to human platelet membrane glycoproteins IIb/IIIa. *Thromb Hemostasis* 1987, 58:243 (Abstr)
38. Barsky SH, Hannah JB: Extracellular hyaline bodies are basement membrane accumulations. *Am J Clin Pathol* 1987, 87:455–460
39. McGeer PL, Zhu SG, Dedhar S: Immunostaining of human brain capillaries by antibodies to very late antigens. *J Neuroimmunol* 1990, 26:213–218
40. Rössler K, Neuchrist C, Kitz K, Scheiner O, Kraft D, Lassmann H: Expression of leukocyte adhesion molecules at the human blood-brain barrier (BBB). *J Neurosci Res* 1992, 31:365–374
41. Faull RJ, Kovach NL, Harlan JM, Ginsberg MH: Stimulation of integrin-mediated adhesion of T lymphocytes and monocytes: two mechanisms with divergent biological consequences. *J Exp Med* 1994, 179:1307–1316
42. Delwel GO, de Melker AA, Hogervorst F, Jaspars LH, Fles DLA, Kuikman I, Lindblom A, Paulsson M, Timpl R, Sonnenberg A: Distinct and overlapping ligand specificities of the $\alpha_3\beta_1$ integrins: recognition of laminin isoforms. *Mol Biol Cell* 1994, 5:203–215
43. Koyama N, Seki J, Vergel S, Mattson EJR, Yednock T, Kovach NL, Harlan JM, Clowes AW: Regulation and function of an activation-dependent epitope of the β_1 integrins in vascular cells after balloon injury in baboon arteries and in vitro. *Am J Pathol* 1996, 148:749–761
44. Pirila L, Heino J: Altered integrin expression in rheumatoid synovial lining type B cells: in vitro cytokine regulation of $\alpha_1\beta_1$, $\alpha_6\beta_1$, and $\alpha_v\beta_5$ integrins. *J Rheumatol* 1996, 23:1691–1698
45. Merrill JE, Benveniste EN: Cytokines in inflammatory brain lesions: helpful and harmful. *Trends Neurosci* 1996, 19:331–338
46. Navikas V, Link H: Review: cytokines and the pathogenesis of multiple sclerosis. *J Neurosci Res* 1996, 45:322–333
47. Woldemar M, Alon R, Springer TA: The C-C chemokine MCP-1 differentially modulates the avidity of β_1 , and β_2 integrins on T lymphocytes. *Immunity* 1996, 4:179–187
48. Furie MB, Randolph GJ: Chemokines and tissue injury. *Am J Pathol* 1995, 146:1287–1301
49. Luster AD, Greenberg SM, Leder P: The IL-10 chemokine binds to a specific cell surface heparan sulfate site shared with platelet factor 4, and inhibits endothelial cell proliferation: *J Exp Med* 1995, 182:219–231
50. Goodkin DE, Rudick RA, Ross JS: The use of brain magnetic resonance imaging in multiple sclerosis. *Arch Neurol* 1994, 51: 505–516
51. Domanico SZ, Pelletier AJ, Havran WL, Quaranta V: Integrin $\alpha_6\beta_1$ induces CD81-dependent cell motility without engaging the extracellular matrix migration substrate. *Mol Biol Cell* 1997, 8:2253–2265
52. Okada Y, Copeland BR, Hamann GF, Koziol JA, Cheresh DA, del Zoppo GJ: Integrin $\alpha_v\beta_3$ is expressed in selected microvessels after focal cerebral ischemia. *Am J Pathol* 1996, 149:37–44
53. Defilippi P, Truffa G, Stefanuto G, Altruda F, Silengo L, Tarone G: Tumor necrosis factor and interferon α modulate the expression of the vitronectin receptor (integrin β_3) in human endothelial cells. *J Biol Chem* 1991, 266:7638–7645
54. Conforti G, Dominguez-Jimenez, Zanetti A, Gimbrone MA, Jr, Cremona O, Marchisio PC, Dejana E: Human endothelial cells express integrin receptors on the luminal aspects of their membrane. *Blood* 1992, 80:437–446
55. Zanetti A, Conforti G, Hess S, Martin-Padura I, Ghibaudi E, Preissner KT, Dejana E: Clustering of vitronectin and RGD peptides on microspheres leads to engagement of integrins on the luminal aspect of endothelial cell membranes. *Blood* 1994, 84:1116–112
56. 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
57. Cannella B, Raine CS: The adhesion molecule and cytokine profile of multiple sclerosis lesions. *Ann Neurol* 1995, 37:424–435
58. Bö L, Peterson JW, Mørk S, Hoffman PA, Gallatin WM, Ransohoff RM, Trapp BD: Distribution of immunoglobulin superfamily members ICAM-1, -2, -3, and the β_2 integrin LFA-1 in multiple sclerosis lesions. *J Neuropathol Exp Neurol* 1996, 55:1060–1072
59. Lampugnani MG, Resnati M, Dejana E, Marchisio PC: The role of integrins in the maintenance of endothelial monolayer integrity. *J Cell Biol* 1991, 112:479–490
60. Defilippi P, van Hinsbergh V, Bertolotto A, Rossino P, Silengo L, Tarone G: Differential distribution and modulation of expression of $\alpha_1\beta_1$ integrin on human endothelial cells. *J Cell Biol* 1991, 114:855–863
61. Esiri MM, Morris CS: Immunocytochemical study of macrophages and microglial cells and extracellular matrix components in human CNS disease. 2. Non-neoplastic diseases. *J Neurol Sci* 1991, 101: 59–72
62. Mori S, Sternberger NH, Herman MM, Sternberger LA: Variability of laminin immunoreactivity in human autopsy brain. *Histochemistry* 1992, 97:237–241
63. Jucker M, Tian M, Ingram DK: Laminins in the adult and aged brain. *Mol Chem Neuropathol* 1996, 28:209–218
64. Bartlett MR, Underwood PA, Parish CR: Comparative analysis of the ability of leucocytes, endothelial cells and platelets to degrade the subendothelial basement membrane: evidence for cytokine dependence and detection of a novel sulfatase. *Immunol Cell Biol* 1995, 73:113–124
65. Harvath L, Brownson NE, Fields GB, Skubitz AP: Laminin peptides stimulate human neutrophil motility. *J Immunol* 1994, 152:5447–5456