Integrin–Matrix Interactions in the Cerebral Microvasculature

Gregory J. del Zoppo, Richard Milner

Abstract—The integrity of all organ systems requires faithful interaction between its component cells and the extracellular matrix (ECM). In the central nervous system (CNS), matrix adhesion receptors are uniquely expressed by the cells comprising the microvascular compartment, and by neurons and their supporting glial cells. Cells within the cerebral microvasculature express both the integrin and dystroglycan families of matrix adhesion receptors. However, the functional significance of these receptors is only now being explored. Capillaries of the cerebral microvasculature consist of the luminal endothelium, which is separated from circumferential astrocyte end-feet by the intervening ECM of the basal lamina. Endothelial cells and astrocytes cooperate to generate and maintain the basal lamina and the unique barrier functions of the endothelium. Integrins and the dystroglycan complex are found on the matrix-proximate faces of both endothelial cells and astrocyte end-feet. Pericytes rest against the basal lamina. In the extravascular compartment, select integrins are expressed on neurons, microglial cells, and oligodendroglia. Significant alterations in both cellular adhesion receptors and their ligands occur under the conditions of focal cerebral ischemia, multiple sclerosis (MS) and the modeled condition experimental autoimmune encephalomyelitis (EAE), certain tumors of the CNS, and arteriovenous malformations (AVMs). The changes in matrix adhesion receptor expression in these conditions support their functional significance in the normal state. We propose that matrix adhesion receptors are essential for the maintenance of the integrity of the blood–brain permeability barrier, and that modulation of these receptors contribute to alterations in the barrier during brain injury. This review examines current information about cell adhesion receptor expression within the cerebral microvasculature and surrounding tissue, and their potential roles during the vascular responses to local injury. (Arterioscler Thromb Vasc Biol. 2006;26:000-000.)

Key Words: blood–brain barrier ▪ cerebral microvasculature ▪ dystroglycan ▪ integrins ▪ matrix adhesion receptors

The cerebral microvasculature is unique in that while serving as a conduit for supplying blood-to-brain structures, it is also completely incorporated within the neuropil, allowing direct interactions with glia and neurons. The cerebral microvasculature is also functionally dynamic. It maintains and with states of arousal and neuronal activation increases local and regional blood flow to provide cellular nutritional support. Both local and distant control of cerebral blood flow during activation results from neuron-vascular coupling via astrocytes, and from direct innervation. The close proximity between the endothelium and astrocyte end-feet of intact cerebral capillaries also implies potential communication between the cells across the basal lamina.

Pial and cortical penetrating arteries consist of an endothelial cell layer, the basal lamina (derived from the extracellular matrix [ECM]), a myointima with smooth muscle cells encased in the matrix, and an adventitia. Arising from the leptomeninges, the adventitia of the cortical penetrating arteries is an extension of the subarachnoid space which forms the Virchow-Robbin's space until it disappears into the glia limitans, the abluminal boundary formed by the astrocyte end-feet in small-caliber microvessels. In the cortical gray matter, the microvasculature consists of hierarchical arrays descending from pial penetrating arteries. In contrast, in the gray matter of the corpus striatum neurons are arranged in a more-or-less consistent and orderly fashion in relation to their adjacent microvessel supply. These arrangements derive from laminin-directed migration of both neurons and microvessel elements during development of the central nervous system (CNS).

Capillaries comprise 60% of the cerebral microvasculature, and are an integral part of the neuropil. Within cerebral capillaries the matrix-containing basal lamina separates the specialized endothelium from astrocyte foot processes. Astrocytes participate in both the capillary ultrastructure and in communication with nearby neurons. Communication among astrocytes follows from their syncytial arrangement.
and occurs via Ca\textsuperscript{2+} channels.\textsuperscript{16} Neuronal stimulation can initiate microvascular and endothelial cell responses via these glial elements.\textsuperscript{17} Nedergaard has shown that neuronal function can also be modulated by astrocyte activation,\textsuperscript{18,19} and that astrocytes can signal.\textsuperscript{20}

In addition to these cellular components of cerebral capillaries, recent evidence suggests the importance of pericytes and microglia to maturation of endothelial cell contacts and to the responses of the neurovascular unit to ischemia.\textsuperscript{21,22} Pericytes are found in the basal lamina matrix on the luminal aspect of astrocyte end-feet.

**Endothelial Transport Properties**

Cerebral capillaries strictly limit transport of solutes, proteins, and chemical agents in the circulation into the neuropil. In particular, activated proteases (eg, thrombin) from the plasma compartment toxic to neurons and supporting cells are prevented from reaching the extravascular space.\textsuperscript{23,24} The capillary endothelium is the primary interface for regulation of the exchange of amino acids, transport of ions via the Na\textsuperscript{+}/K\textsuperscript{+}/Cl\textsuperscript{−} co-transporter, the Na+/H\textsuperscript{+} exchanger, the inward rectifying K\textsuperscript{+} channel, and other ports, the entry of glucose via its transporters, and the transport of fatty acids and lysophosphatidylcholine from the plasma.\textsuperscript{25} These ports regulate the ionic milieu of the astrocyte end-feet, and the energy supply for the neuropil.

**Specialization of the Microvasculature**

Regional specialization of cerebral microvascular endothelial cell function exists along the microvessel axis.\textsuperscript{26,27} Spatz et al have shown that under normal conditions glucose and amino acid transporter expression varies with the microvessel diameter.\textsuperscript{28,29} During local inflammation, adhesion receptors for leukocyte adhesion are expressed predominantly by the post-capillary venule endothelium.\textsuperscript{30,31} In view of both regional and individual specialization of neurons, local subspecialization of the neuron-microvessel relationship in cerebral microvascular beds seems possible, although it is as yet unproven. Another reflection of this specialization is the variation in expression of adhesion receptor types and their matrix ligands in the cerebral microvasculature.\textsuperscript{32}

**The Cerebral Microvascular Permeability Barrier**

The functional barrier properties of cerebral microvessels are represented by 2 structures: (1) the cohesive and resistance properties of the endothelial cells, involving the inter-endothelial tight junctions (TJs) and the extracellular matrix (ECM) components of the basal lamina (Figure 1). Both the endothelial cell permeability barrier and the basal lamina matrix derive from cooperation between the endothelium and astrocytes, which together constitute the blood–brain barrier.\textsuperscript{7,33–35} Recent data suggest that the endothelial cell barrier properties are only partly explained by tight junction proteins,\textsuperscript{36–40} and that astrocyte adhesion is required to regulate the quality of the interendothelial barrier.\textsuperscript{41–42} Although attention has been focused increasingly on the tight junction apparatus, the stability of the endothelial cell–astrocyte configuration requires matrix adhesion. In this context, focal ischemia leads to abrupt and significant alterations in the matrix constituents, changes in matrix adhesion receptor expression by both endothelial cells and astrocytes, and to local increases in vascular permeability.

**Matrix Adhesion Receptors**

The integrins and dystroglycan constitute 2 well-characterized families of the cellular–ECM adhesion receptors associated with the microvasculature in the CNS.

**Integrins**

Integrins are cell surface transmembrane, noncovalently-linked αβ heterodimers that recognize specific matrix ligands.\textsuperscript{43} Functionally, integrins can regulate cell behavior by:
integrin receptors, including laminin as the primary matrix ligand with a number of laminin-membrane subunit, binds to the ECM proteins laminin (the between the intracellular cytoskeleton and the ECM. The receptor, distinct from integrins, that forms a physical link which are also expressed in the CNS microvasculature.54–57

Dystroglycan
Dystroglycan is a single αβ heterodimeric transmembrane receptor, distinct from integrins, that forms a physical link between the intracellular cytoskeleton and the ECM. The α subunit is formed by proteolytic cleavage of a single precursor, and interacts noncovalently with the 43-kDa transmembrane β subunit to form the active receptor.46,47 α-dystroglycan, the 120- to 190-kDa glycosylated extracellular subunit, binds to the ECM proteins laminin (the laminin-α1 chain), perlecian (heparin sulfate proteoglycan [HSPG]), and agrin.48,49 The intracellular carboxy-terminus of β-dystroglycan binds to the cytoskeletal proteins dystrophin and utrophin.50,51 Expression of αβ-dystroglycan in the cerebral microvasculature is associated with both endothelial cells and astrocytes.49,52,53 The dystroglycan complex shares laminin as the primary matrix ligand with a number of integrin receptors, including α1β1, α2β1, α3β1, and α4β1, which are also expressed in the CNS microvasculature.54–57

Roles of Integrin Receptors in the CNS as Defined by Integrin Null Mice
Because the absence of integrin subunit α1, α2, α3, or β1 expression results in embryonic lethality in murine knockout constructs (Table 1),58–61 it has not been established whether their functions are required for maintaining blood–brain barrier integrity. Deletions of the integrin subunits α5, α6, α8, β8, or β8 are lethal in the perinatal period in mice.62–66 Significantly, deletions of the integrin subunits α6, α8, and β8 demonstrate a clear CNS phenotype.64,66,67

α6 Integrin
The premature death of α6 integrin null mice is caused by defective attachment of the epidermis to the matrix of the underlying basement membrane (reminiscent of the disorders junctional epidermolysis bullosa and bullous pemphigoid in humans).64,65 Mice lacking the integrin α6 subunit also show defective neuronal migration in the CNS. Neurons migrate beyond their normal position resulting in disordered layering of the cerebral cortex and neuronal ectopia on the outer surface of the cortex.65 A similar phenotype is generated by deletion within the laminin γ1 chain gene and the gene of the ECM protein reelin.66,67 demonstrating that laminin α6β1 signaling is required for the establishment of neuronal patterning during cerebral development. The impact on cerebrovascular development has not been investigated.

α8 or β8 Integrins
Within the endothelium or microvascular structures associated with the endothelium depend on integrin α8β8 in a way that blood vessels outside the CNS do not.70 Mice which lack the α8 or β8 integrin subunits suffer cerebral hemorrhage, and do not survive past the perinatal period.64,66 Cerebral vessels in α8 or β8 null constructs dilate at an early developmental stage resulting in a leaky vasculature. This defect has been attributed to failure of proper adhesive interactions among the neuroepithelial cells, astrocytes, and endothelial cells during cerebrovascular development. Conditional

<table>
<thead>
<tr>
<th>Integrin Subunit</th>
<th>Viability</th>
<th>Fertility</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1</td>
<td>+</td>
<td>+</td>
<td>Defects in bone healing and reduced tumor angiogenesis</td>
<td>123</td>
</tr>
<tr>
<td>α2</td>
<td>+</td>
<td>+</td>
<td>Reduced branching morphogenesis and platelet adhesion</td>
<td>124</td>
</tr>
<tr>
<td>α3</td>
<td>Perinatal lethal</td>
<td>+</td>
<td>Kidney, lung, and skin defects</td>
<td>62</td>
</tr>
<tr>
<td>α4</td>
<td>Embryonic lethal</td>
<td>−</td>
<td>Placental and heart defects</td>
<td>58</td>
</tr>
<tr>
<td>α5</td>
<td>Embryonic lethal</td>
<td>−</td>
<td>Mesodermal and vascular defects</td>
<td>59</td>
</tr>
<tr>
<td>α6</td>
<td>Perinatal lethal</td>
<td>+</td>
<td>Epidermal detachment, defect in neurogenesis</td>
<td>63</td>
</tr>
<tr>
<td>α7</td>
<td>+</td>
<td>+</td>
<td>Muscular dystrophy</td>
<td>125</td>
</tr>
<tr>
<td>α8</td>
<td>Perinatal lethal</td>
<td>+</td>
<td>Kidney defect</td>
<td>60</td>
</tr>
<tr>
<td>α9</td>
<td>Perinatal lethal</td>
<td>+</td>
<td>Chylothorax (defect in lymphatic drainage)</td>
<td>126</td>
</tr>
<tr>
<td>α10</td>
<td>Embryonic and perinatal lethal</td>
<td>+</td>
<td>Cerebral hemorrhage</td>
<td>64</td>
</tr>
<tr>
<td>β1</td>
<td>Embryonic lethal</td>
<td>−</td>
<td>Fails to gastrulate</td>
<td>61, 64</td>
</tr>
<tr>
<td>β2</td>
<td>+</td>
<td>+</td>
<td>Leukocyte adhesion deficiency</td>
<td>127</td>
</tr>
<tr>
<td>β3</td>
<td>+</td>
<td>+</td>
<td>Platelet defect</td>
<td>128</td>
</tr>
<tr>
<td>β4</td>
<td>Perinatal lethal</td>
<td>+</td>
<td>Epidermal detachment</td>
<td>65</td>
</tr>
<tr>
<td>β5</td>
<td>+</td>
<td>+</td>
<td>Accelerated age-related blindness</td>
<td>129</td>
</tr>
<tr>
<td>β6</td>
<td>+</td>
<td>+</td>
<td>Inflammation in skin and lungs</td>
<td>130</td>
</tr>
<tr>
<td>β7</td>
<td>+</td>
<td>+</td>
<td>Gut-associated lymphocyte defects</td>
<td>131</td>
</tr>
<tr>
<td>β8</td>
<td>Embryonic and perinatal lethal</td>
<td>+</td>
<td>Cerebral hemorrhage</td>
<td>66</td>
</tr>
</tbody>
</table>
knockout preparations which specifically remove $\alpha$ integrins from either endothelial cells or astrocytes have demonstrated that integrin $\alpha_\beta$ expressed by astrocytes is important for the adhesive interaction to take place.$^{71,72}$ Recent work has suggested that astrocyte $\alpha_\beta$ integrin may activate transforming growth factor-$\beta$ and thereby stabilize the endothelium, such that in the absence of this integrin, the microvasculature is more prone to hemorrhage.$^{73}$

$\beta_1$ Integrins

The role of $\beta_1$ integrins in neurons and glia has been evaluated using cre-lox technology. Such cell-selective knockouts produce a phenotype similar to deletions of the $\alpha$ integrin, $\gamma$-laminin, and reelin genes.$^{63,68,69}$ Integrin $\beta_1$-/- neurons adhere to and migrate along radial glia normally, but result in a disordered neuronal layering of the cerebral cortex.$^{74}$ Furthermore, glial end-feet fail to make proper connections to the meningeal basement membrane, disordering the marginal zone of the developing cerebral cortex and resulting in aberrant cortical organization. Hence, while integrin expression on both endothelial cells and astrocytes after focal cerebral ischemia accompanies structural alterations in the microvasculature,$^{75}$ this suggests essential roles for $\beta_1$ integrins within the microvasculature.

Regulation of Integrin Expression and Function During CNS Development

Integrin function is regulated by the level of cell surface expression and by the level of integrin activation. For example, in the developing CNS, retinal ganglion axons adhere to and extend on laminin until they reach the tectum, where they detach from laminin.$^{76}$ This is caused by both decrease in the axonal surface expression of integrin $\alpha_\beta$, and loss of the active conformation of the $\beta_1$ integrins still expressed on the cell surface of retinal ganglion cells.$^{77,78}$ Another form of developmental integrin regulation is exemplified by oligodendroglial cells which change the $\beta$-subunit partners of $\alpha$ integrins from $\alpha_\beta$ to $\alpha_\beta$ during terminal differentiation.$^{79}$

Cerebral blood vessel maturation is associated with marked up-regulation of $\beta_1$ integrin expression during CNS development,$^{80}$ which coincides with a switch in endothelial cell $\beta_1$ integrin expression. In the postnatal angiogenic period cerebral endothelial cells express the fibronectin receptors $\alpha_\beta$ and $\alpha_\beta$, but after cessation of angiogenesis, switch to the laminin receptors $\alpha_\beta$ and $\alpha_\beta$. This “integrin switch” coincides with a concomitant change in the endothelial cell–matrix ligands from fibronectin to laminin. Taken together, this suggests an instructive role for $\beta_1$ integrins during angiogenesis in the CNS.

Adhesion Receptor Expression in the Adult Brain

The constitutive high level expression of select integrins and dystroglycan within the cerebral capillaries in mature adult brain suggest active links of endothelial cells and astrocyte end-feet to the intervening basal lamina matrix (Table 2).$^{32,81–83}$

Endothelial Cells

$\beta_1$ Integrins

Haring et al characterized microvascular integrin subunit expression in the normal primate striatum and cortex gray matter,$^{33}$ in part confirming other reports of vascular integrin expression in human brain (Table 2).$^{81–83}$ Integrin subunits $\alpha_1$ and $\alpha_6$ are distributed throughout the normal cerebral microvasculature in a pattern identical to subunit $\beta_1$, suggesting the common expression of integrins $\alpha_\beta$ and $\alpha_\beta$ throughout the cerebrovascular tree in all mammalian species.$^{80}$ Their expression parallels that of $\beta_1$-matrix ligands laminin, collagen IV, and fibronectin. The integrin $\alpha_4$ subunit is expressed on a subset of capillaries less frequently than $\alpha_1$ or $\alpha_6$. The reasons for this differential expression are not known.$^{81}$ McGeer et al and Paulus et al confirmed that integrin $\alpha_1$ appears on the cerebral microvasculature, and Haring et al noted its expression by non-capillary microvessels exclusively in a pattern quite similar to subunits $\alpha_4$, $\alpha_5$, and $\beta_1$.$^{81,82}$ The expression of several known typical $\alpha$-subunit partners for the integrin $\beta_1$ on select capillary/ microvessel subclasses might be explained by endothelial cell specialization along the microvascular axis.

$\alpha_1\beta_1$ Integrins

Integrin subunits $\alpha_1$ and $\beta_1$ are roughly equally expressed on a very small proportion of resting non-capillary microvessels in comparison to the distribution of basal lamina matrix proteins.$^{32,44}$ Integrin $\alpha_1\beta_1$ expression is significantly upregulated after the onset of focal ischemia.$^{85}$

Glial Cells

$\beta_1$ Integrins

The observation of $\beta_1$ integrin expression on astrocytes is consistent in post-mortem human brain and rat brain.$^{82,83}$ Both subunits $\alpha_1$ and $\beta_1$ are found on astrocyte fibers surrounding select microvessels in the adult primate.$^{32,86}$ In addition, murine primary astrocytes in culture express integrins $\alpha_1\beta_1$, $\alpha_1\beta_1$, $\alpha_1\beta_1$, and $\alpha_1\beta_1$. Function-blocking studies show that these integrins are functionally active adhesion receptors for laminin ($\alpha_1\beta_1$, $\alpha_1\beta_1$, and $\alpha_1\beta_1$) and fibronectin ($\alpha_1\beta_1$).$^{88,89}$ Astrocytes in culture also express integrins $\alpha_1\beta_1$ and $\alpha_1\beta_1$ which, on a vitronectin substrate, promote astrocyte adhesion and migration, respectively.$^{89}$

$\alpha_4\beta_1$ Integrins

Integrin $\alpha_4\beta_1$ is expressed on the astrocyte end-feet in a small proportion of normal capillaries and noncapillary microvessels (Table 2).$^{32,86}$ The reasons for this restricted expression are so far unknown. A matrix ligand for integrin $\alpha_4\beta_1$, laminin-5 is codistributed in the cerebral microvascular basal lamina with the major matrix constituents laminin-1, collagen type IV, and cellular fibronectin.$^{80}$ Hemidesmosomes, which anchor epithelial cells to the cuticular basement membrane, contain the integrin $\alpha_4\beta_1$, and have been found in astrocyte end-feet at the vascular basal lamina interface of larger
In short, integrin αβ could play active roles in the attachment of astrocyte end-feet to the basal lamina of select microvessels for maintaining their close apposition to the endothelium.

Other Integrin Receptors

**α4 and α6 Integrins**

While α4 and α6 integrins are expressed during angiogenesis, they are developmentally downregulated after angiogenesis and are expressed by only a small proportion of noncapillary cerebral microvessels in the primate. But, subunit α5, and not α6, has been identified on adult human cerebral microvessels in one report.

**Vascular Matrix-Adhesion Receptor Expression During Focal Cerebral Ischemia**

The rather static ultrastructural features of the cerebral microvasculature belie considerable local reactivity. Experimental preparations demonstrate that within the core regions of ischemic injury the apparently stable microvasculature responds rapidly after occlusion of a brain-supplying artery. For instance, changes in microvessel integrin expression occur within 1 to 2 hours in the ischemic core, and are accompanied by detachment of astrocyte end-feet, local loss of permeability barrier integrity with edema accumulation, and the appearance of markers of angiogenesis (Table 2). The endothelial cell-associated leukocyte adhesion receptors P-selectin and intercellular adhesion molecule-1 also appear in this time frame, followed by the expression of E-selectin.

The changes seen in microvascular integrin expression accompany rapid local alterations in their matrix ligands within the basal lamina. Laminin-1, collagen type IV, and cellular fibronectin decrease to 60% of the baseline in the ischemic core by 24 hours following middle cerebral artery occlusion (MCAO). Within 2 hours there is significantly greater loss of the HSPG perlecan than laminin within the basal lamina, indicating the greater sensitivity of this integrin β1-ligand to focal ischemia. These events correlate with the simultaneous generation of the matrix proteases pro-matrix metalloproteinase (MMP)-2 (pro-MMP-2), pro-MMP-9, and their activators urokinase, membrane-type 1 (MT1)-MMP, and MT3-MMP on microvessels (and neurons) within the regions of injury. Cysteine protease activity, marked by the appearance of cathepsin L, corresponds to the loss of HSPG. Importantly, loss of the major basal lamina proteins are directly associated with extravasation of erythrocytes and hemorrhagic transformation.

**Endothelial Cells**

**β1 Integrins**

While the endothelium remains intact, glial end-feet are displaced from the basal lamina. By 2 hours after MCAO, endothelial cell β1-integrin expression by the endothelium is lost in 69±7% of microvessels within the ischemic core (Figure 2). This loss is sustained and does not recover despite restitution of flow. The mechanisms by which microvessels. In short, integrin αβ could play active roles in the attachment of astrocyte end-feet to the basal lamina of select microvessels for maintaining their close apposition to the endothelium.

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**TABLE 2. Cellular Changes and Integrin Expression Levels on Cerebral Capillaries in Disease States.**

<table>
<thead>
<tr>
<th>Capillary Component</th>
<th>Normal</th>
<th>Focal Cerebral Ischemia</th>
<th>Multiple Sclerosis/EAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial cell</td>
<td></td>
<td>Increased barrier permeability</td>
<td>Increased barrier permeability</td>
</tr>
<tr>
<td>α1β1</td>
<td>+</td>
<td>↓ 75</td>
<td>↑ 106</td>
</tr>
<tr>
<td>α3β1</td>
<td>+</td>
<td>↓ 75</td>
<td>= 105</td>
</tr>
<tr>
<td>α6β1</td>
<td>+</td>
<td>↓ 75</td>
<td>↓ 105</td>
</tr>
<tr>
<td>αvβ3</td>
<td>-</td>
<td>↑ 84.85</td>
<td>↑ 105</td>
</tr>
<tr>
<td>αβ-dystroglycan</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Astrocyte: end-foot</td>
<td></td>
<td>Detachment of end-feet</td>
<td>Unknown</td>
</tr>
<tr>
<td>α6β4</td>
<td>+</td>
<td>↓ 86</td>
<td>↑ 106</td>
</tr>
<tr>
<td>αβ-dystroglycan</td>
<td>+</td>
<td>?</td>
<td>↓ 107</td>
</tr>
<tr>
<td>Astrocyte: fiber</td>
<td></td>
<td>Intact</td>
<td>Intact</td>
</tr>
<tr>
<td>α1β1</td>
<td>+</td>
<td>↓ 75</td>
<td>?</td>
</tr>
<tr>
<td>α4</td>
<td>-</td>
<td>-</td>
<td>↑ 106</td>
</tr>
<tr>
<td>Matrix</td>
<td></td>
<td>Decreased density</td>
<td>Mixed response</td>
</tr>
<tr>
<td>Laminins</td>
<td>+</td>
<td>↓ 15.86</td>
<td>?</td>
</tr>
<tr>
<td>Collagen type IV</td>
<td>+</td>
<td>↓ 15</td>
<td>?</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>+</td>
<td>↓ 15.15</td>
<td>↑ 132,135</td>
</tr>
<tr>
<td>Perlecan (HSPG)</td>
<td>+</td>
<td>↓ 84</td>
<td>?</td>
</tr>
<tr>
<td>Vitronectin</td>
<td>-</td>
<td>?</td>
<td>↑ 133,134</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>-</td>
<td>?</td>
<td>↑ 135,136</td>
</tr>
</tbody>
</table>

*Fibronectin of cellular origins.
†Blood-derived fibronectin
ischemia diminishes microvessel $\beta_1$ expression are not yet known. However, within the ischemic core, downregulation of microvessel-associated integrin $\beta_1$ gene appears heterogeneous: confluent regions of increased $\beta_1$ transcription on microvessels surround subregions with depressed $\beta_1$ expression early after MCAO. The upregulation of $\beta_1$ in the boundary between the ischemic core and the intermediate affected peripheral zone, and around subregions lacking expression, is consistent with the notion that the “ischemic penumbra” is initially interspersed among relatively unaffected tissue within the core. In time all $\beta_1$ gene expression ceases as the injured subregions merge.

Within the ischemic core, the subunits $\alpha_6$, $\alpha_5$, and $\alpha_4$ are significantly depressed in parallel with the $\beta_1$ subunit (Table 2, Figure 3), suggesting that all endothelial cell $\beta_1$-integrin complexes are affected by ischemia. Endothelial $\alpha_6\beta_1$ expression continues on microvessels which retain laminin within the intact basal lamina; the loss of this integrin generally precedes that of laminin. But, there is no evidence of endothelial cell detachment early after MCAO. The relationships of these changes to local flow conditions are not yet known.

**Integrin $\alpha_6\beta_1$**

Capillary bud formation appears by 7 days after MCAO. However, it is not yet known what roles integrin $\alpha_6\beta_1$ plays in cerebral angiogenesis. Tissue culture studies have shown that integrin $\alpha_6\beta_1$ is expressed by cerebral endothelial cells, but not astrocytes. While $\alpha_6\beta_1$ is not normally expressed by resting endothelial cells in vivo, it is induced during angiogenesis or in cell culture, and plays an important role in mediating brain capillary endothelial cell adhesion to fibronectin, thus promoting endothelial cell survival and proliferation. Focal ischemia stimulates the consistent and significant early expression of the integrin $\alpha_6\beta_1$ on activated noncapillary cerebral microvessels, whereas integrin $\alpha_6\beta_1$ expression is not affected (Table 2, Figure 3). However, the molecular and vascular consequences of coordinated vascular endothelial growth factor (VEGF) and integrin $\alpha_6\beta_1$ expression on activated microvessels, a highly significant relationship, seen by 2 hours after MCAO have not yet been defined. Several model systems have demonstrated local increases in hypoxia inducible factor (HIF)-1α or the cytokines interleukin (IL)-1β and tumor necrosis factor-α, both promoters of VEGF gene expression, in the regions of ischemic injury. Subunit $\alpha_6$ transcription is upregulated along with VEGF in affected microvessels within the ischemic core. Also, a highly significant relationship between microvessel $\alpha_6\beta_1$ expression and fibrin deposition (one of its ligands) within the microvasculature has been observed. The significant relationship among microvascular proliferating cell nuclear antigen, VEGF, and integrin $\alpha_6\beta_1$ expression is independent of time, reflecting the heterogeneity of the evolving injury in the striatum.

**Glial Cells**

**Integrin $\alpha_6\beta_1$**

After the onset of focal ischemia, integrin $\alpha_6\beta_1$ is rapidly lost from the astrocyte end-feet of select microvessels. This corresponds to the separation of astrocytes from the vascular matrix early after MCAO, and to the cell swelling and loss in cytoplasmic density, which accompanies astrocyte separation (Figure 2). These changes in ultrastructure and integrin $\alpha_6\beta_1$ expression also imply fundamental alterations in the end-foot apparatus.

**Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis**

Considerably less is known about the fate of matrix adhesion receptors in the development of lesions in multiple sclerosis (MS). Integrin $\alpha_6\beta_1$ expression on microvascular endothelial cells is altered in MS (Table 2). During the active phase of MS, the expressions of the integrin $\beta_1$ and $\alpha_6$ subunits are
Targeting Integrin–Matrix Interactions as Potential Treatments

To determine the roles of specific integrins in the CNS, function-blocking reagents including monoclonal antibodies and peptides, have been used. Because the importance of integrin-matrix interactions in the regulation of cerebral vascular permeability is only now being realized, specific function-blocking studies have not yet addressed the role of these adhesive events in the integrity of the blood–brain barrier. However, so far 2 nonbarrier integrin-mediated events have been examined in neurological disease: (1) leukocyte infiltration into the neuropil and (2) platelet activation during stroke.

Leukocytes enter the neuropil during the cellular inflammatory phases of MS and ischemic stroke. In murine EAE, α4 integrins and to a lesser extent the β3 integrins on leukocytes mediate their adhesion to the endothelium and transmigration. Based on promising data from animal studies, clinical trials have established that infusion of a humanized monoclonal antibody against the α4 integrin subunit into patients with the relapsing–remitting form of MS significantly reduces both the relapse frequency and the number of new demyelinated lesions. However, total blockade of leukocyte entry into the CNS might not be a desirable outcome for the patient. More effective might be titration of the excessive leukocyte response while leaving some endogenous protection intact.

Blockade of leukocyte β3-integrins early during MCAO significantly decreases focal “no-reflow” in primates. Furthermore, blockade of the platelet-fibrinogen receptor αIIbβ3 increases microvessel patency and decreases injury volume after MCAO in a separate experimental system. A significant dose-dependent increase in debilitating hemorrhage within the ischemic regions can occur with these agents. In keeping with the observation that specific integrin αIIbβ3 blockade can produce hemorrhage within the ischemic territory in animal models, a prospective phase III trial of the pan-β3 inhibitor of platelet αIIbβ3 in ischemic stroke patients has been terminated because of safety concerns. This suggests that despite salutary effects of integrin blockade at the vascular interface a more complete understanding of the mechanisms whereby adhesive events regulate blood–brain barrier integrity and leukocyte trafficking across the microvessel permeability barrier is required.

Adhesion Receptors and the Permeability Barrier

Those observations suggest a need to examine CNS barrier function more carefully. Alterations in endothelial cell and astrocyte adhesion receptor expression within cerebral microvessels coincide with neuronal injury, glial activation, and breakdown of the blood–brain permeability barrier. Current views of blood–brain barrier integrity, altered during focal ischemia and inflammation (eg, MS), have largely ignored the roles of microvessel endothelial cell-matrix-astrocyte adhesion interactions, but mostly focus on interendothelial cell cohesion.

Three lines of evidence suggest that matrix adhesion receptors may play an important role in maintaining the permeability barrier of the brain. First, select integrins and dystroglycan are expressed at high levels specifically at the blood–brain barrier. Second, alterations in the expression of these receptors coincide with breakdown of the permeability barrier, leading to glial activation and neuronal injury. Third, studies of transgenic mice show that the absence of specific integrins leads to breakdown of the cerebral vasculature, supporting the notion that ECM receptors could participate in the maintenance of cerebrovascular integrity.

Based on those observations, we propose that the microvessel permeability barrier in the brain consists of both “horizontal” and “vertical” components. The tight junctions that form between adjacent endothelial cells have been viewed as the basis for the endothelial permeability barrier in mammalian brain. The tight junctions and the interendothelial adherens complexes together constitute the “horizontal” component. We wish to extend this model to include a vertical component, consisting of the matrix adhesion complexes formed between adhesion receptors on both endothelial cells and astrocytes, which anchor the cells to the intervening basal lamina of extracellular matrix. “Vertical” adhesion of the astrocyte foot processes by matrix receptors maintains the close proximity of the astrocyte portion of the microvascular compartment to the endothelium, and hence their contribution to endothelial cell barrier integrity. This proximity is necessary for the competence of the microvascular barrier and its unique resistance properties in the CNS. One important implication of this concept is that by altering vertical adhesion at the barrier interface new avenues of therapeutic potential could be devised.

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Disclosures

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References


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Integrin-Matrix Interactions in the Cerebral Microvasculature
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