Vascular matrix adhesion and the blood–brain barrier

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Abstract

The integrity of the cerebral microvasculature depends on the interaction between its component cells and the extracellular matrix, as well as reorganized cell–cell interactions. In the central nervous system, matrix adhesion receptors are expressed in the microvasculature and by neurons and their supporting glial cells. Cells within cerebral microvessels express both the integrin and dystroglycan families of matrix adhesion receptors. However, the functional significance of these receptors is only now being explored. Endothelial cells and astrocytes within cerebral capillaries co-operate 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 matrix ligands occur during focal cerebral ischaemia, which 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 contributes to alterations in the barrier during brain injury.

Introduction

The microvasculature of the CNS (central nervous system) is structurally and functionally unique. 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. Pial and cortical penetrating arteries consist of an endothelial-cell layer, the basal lamina [derived from the ECM (extracellular matrix)], a myointima with smooth-muscle cells encased in the matrix, and an adventitia. The adventitia of the cortical penetrating arteries arises from the leptomeninges, and together with the subarachnoid space forms the Virchow–Robbins space until it disappears into the glia limitans, the abluminal boundary formed by the astrocyte end-feet in small calibre microvessels [1,2].

In the cortical grey matter, the microvasculature consists of hierarchical arrays descending from pial penetrating arteries [3,4]. In the striatal grey matter, however, neurons are arranged in a highly ordered fashion in relation to their adjacent microvessel supply [5]. In this network, capillaries branch at 30–40 µm intervals, allowing ready redirection of flow (G.J. del Zoppo, unpublished work). These

Key words: blood–brain barrier, dystroglycan, extracellular matrix, focal ischaemia, integrin, matrix adhesion receptor.

Abbreviations used: CNS, central nervous system; ECM, extracellular matrix; MCAO, middle cerebral artery occlusion; MMP, matrix metalloproteinase; MT1, membrane-type 1; VEGF, vascular endothelial growth factor.

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arrangements derive from laminin-directed migration of both neurons and microvessel elements during development of the CNS [6,7].

Capillaries comprise $\geq$60% of the cerebral microvasculature, and are an integral part of the neuropil. Within cerebral capillaries, the matrix-containing basal lamina separates the specialized endothelium from astrocyte foot processes [8]. Astrocytes participate in both the capillary ultrastructure and in communication with nearby neurons. Communication among astrocytes follows from their syncytial arrangement and occurs via Ca$^{2+}$ channels [9]. Neuronal stimulation can initiate microvascular and endothelial-cell responses via these glial elements [10]. Nedergaard and co-workers have shown that neuronal function can also be modulated by astrocyte activation [11,12] and that astrocytes can signal [13].

In addition to the close proximity of endothelial cells and astrocytes, recent evidence suggests the importance of pericytes and microglia for maturation of endothelial-cell contacts within cerebral capillaries and to the responses of the neurovascular unit to ischaemia [14,15]. Pericytes are found in the basal lamina matrix on the luminal aspect of astrocyte end-feet.

The cerebral microvasculature is functionally dynamic. It maintains and, with states of arousal and neuronal activation, increases local and regional blood flow to provide cellular nutritional support [16,17]. Both local and distant control of cerebral blood flow during activation results from neurovascular coupling via astrocytes, and from direct innervation [16]. The close proximity between the endothelium and astrocyte end-feet of intact cerebral capillaries also implies potential communication between these cells across the basal lamina.

**Endothelial transport properties**

Cerebral capillaries strictly limit the transport of solutes, proteins and chemical agents from entry into the neuropil. Activated proteinases (e.g. thrombin) from the plasma compartment toxic to neurons and supporting cells are prevented from reaching the extravascular space [18,19] and are inhibited at the capillary interface locally (e.g. perivascular proteinase nexin-1) [20]. The capillary endothelium is the primary interface for regulation of the exchange of amino acids, transport of ions via the Na$^+$/K$^+$–Cl$^-$ co-transporter, the Na$^+$/H$^+$ exchanger, the inward rectifying K$^+$ channel, and other ports, the entry of glucose via its transporters, and the transport of fatty acids and lysophosphatidylcholine from the plasma [21]. These ports regulate the ionic milieu of the astrocyte end-feet, and the energy supply for the neuropil. A recent report indicates the potential role of a sulfonylurea–the Na$^+$/H$^+$ exchanger, the inward rectifying K$^+$ channel, and other ports, the entry of glucose via its transporters, and the transport of fatty acids and lysophosphatidylcholine from the plasma [21]. These ports regulate the ionic milieu of the astrocyte end-feet, and the energy supply for the neuropil. A recent report indicates the potential role of a sulfonylurea channel on endothelial cells and astrocytes

**Specialization of the microvasculature and the permeability barrier**

Regional specialization of the cerebral microvascular endothelium exists along the microvessel axis, which can also be modulated by ischaemia [23]. Spatz and co-workers [23] have shown that under normal conditions glucose and amino acid transporter expression varies with the microvessel diameter. During focal ischaemia and local inflammation, receptors for leucocyte adhesion and transmigration are expressed predominantly by the post-capillary venule endothelium [24,25]. In view of both regional and individual specialization of neurons, it is plausible that local sub-specialization of the neuron–microvessel relationship in cerebral microvascular beds could develop, although so far this is unproven. Another reflection of this specialization is the variation in expression of adhesion receptor types and their matrix ligands in the cerebral microvasculature (see below) [26].

The functional (‘blood–brain’) barrier properties of cerebral microvessels depend on: (i) the cohesive and resistance properties of the endothelial cells, and (ii) the intact basal lamina. Both derive from cooperation between the endothelium and astrocytes, which together constitute the blood–brain barrier [22,27,28]. Recent results suggest that the endothelial–cell barrier properties are only partly explained by tight junction proteins [29–32] and that astrocyte adhesion is required to regulate the quality of the inter-endothelial barrier [32,33]. The resistance properties involve the interendothelial tight junction proteins [ZO (zonula occludens)-1 protein, claudin-5 and occludins], junctional adhesion molecules, and the adherens complex E-cadherin. 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 ischaemia initiates abrupt and significant alterations in microvascular matrix, changes in matrix adhesion receptor expression by both endothelial cells and astrocytes and local increases in vascular permeability. These events are undoubtedly complex, but the requirements for matrix adhesion receptor integrity appear central.

**Matrix adhesion receptors**

Members of the integrin and dystroglycan cellular–ECM adhesion receptor families are associated with the microvasculature in the CNS. Both integrin and dystroglycan receptor family members are essential for organ development, and particularly for proper organization and function of the CNS (Table 1) [34].

**Integrins**

Integrins are cell-surface transmembrane, non-covalently-linked $\alpha\beta$ heterodimers that recognize specific matrix ligands [35]. Integrins are central to many developmental, physiological and pathological processes of the CNS and other tissues [34]. Functionally, these receptors can regulate cell behaviour by (i) forming a transmembrane link between the matrix and the cell cytoskeleton, (ii) transducing extracellular stimuli to intracellular signals, and (iii) generating increased receptor specificity by cellular activation. In the adult CNS, integrin subunits are distributed in the microvasculature in distinct expression patterns [26].
Cerebral blood vessel maturation is associated with marked up-regulation of β1-integrin expression during CNS development, which coincides with a switch in endothelial-cell β1-integrin expression. The loss of β1-integrin expression on both endothelial cells and astrocytes following focal cerebral ischaemia accompanies structural alterations in the microvasculature [36]. Deletion of the integrin subunits α6, αv or β8 results in embryonic or perinatal lethality, but also in the case of the latter two receptors there is evidence of cerebral haemorrhage [37]. This has been interpreted as evidence of participation in cerebral vascular development. Interestingly, 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.

### Dystroglycan

Dystroglycan is a single αβ heterodimeric transmembrane receptor, distinct from integrins, that links the intracellular cytoskeleton of select cells with the ECM [38]. α-Dystroglycan, the 120–190 kDa glycosylated extracellular subunit, binds to the ECM proteins laminin (the laminin α2 chain), perlecan and agrin [39]. The intracellular C-terminus of β-dystroglycan binds to the cytoskeletal proteins dystrophin and utrophin. Expression of αβ-dystroglycan in the cerebral microvasculature is associated with both endothelial cells and astrocytes [40]. The dystroglycan complex shares laminin as the primary matrix ligand with a number of integrin receptors, including α1β1, α3β1, α6β1 and α6β4 [41,42].

Dystroglycan has been described on the foot processes of astrocytes in the microvasculature and on pial surfaces, and in ‘neuronal elements’ of the hippocampus and cerebellar cortex in the mouse [43]. A conditional knockout of α-dystroglycan tied to an astrocyte-specific promoter was associated with discontinuities in the glia limitans and a type of glial hypertrophy [43]. This suggests the participation of α-dystroglycan in cerebral and possibly cerebral microvascular development.

### Table 1 | Changes in cerebral capillary matrix adhesion receptor relationships

<table>
<thead>
<tr>
<th>Capillary component</th>
<th>Normal</th>
<th>Focal cerebral ischaemia</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial cell</td>
<td></td>
<td>Increased barrier permeability</td>
<td>[36]</td>
</tr>
<tr>
<td>α1β1</td>
<td>+</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>α3β1</td>
<td>+</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>α6β1</td>
<td>+</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>αvβ3</td>
<td>-</td>
<td>↑</td>
<td>[51,58]</td>
</tr>
<tr>
<td>αβ-Dystroglycan</td>
<td>+</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>Astrocyte: end-foot</td>
<td>End-foot detachment and swelling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α6β4</td>
<td>+</td>
<td>↓</td>
<td>[46]</td>
</tr>
<tr>
<td>αβ-Dystroglycan</td>
<td>+</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>Astrocyte: fibres</td>
<td>Intact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α1β1</td>
<td>+</td>
<td>↓</td>
<td>[36]</td>
</tr>
<tr>
<td>αv</td>
<td>-</td>
<td>-</td>
<td>–</td>
</tr>
<tr>
<td>Matrix</td>
<td>Decreased density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminins</td>
<td>+</td>
<td>↓</td>
<td>[8,46]</td>
</tr>
<tr>
<td>Collagen type IV</td>
<td>+</td>
<td>↓</td>
<td>[8]</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>+</td>
<td>↓</td>
<td>[8]</td>
</tr>
<tr>
<td>Perlecan (HSPG)</td>
<td>+</td>
<td>↓</td>
<td>[53]</td>
</tr>
<tr>
<td>Vitronectin</td>
<td>-</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>-</td>
<td>?</td>
<td>–</td>
</tr>
</tbody>
</table>

*Fibronectin of cellular origin.

The expression of β1-integrins on astrocytes is consistent in post-mortem human brain and rat brain [45]. Both subunits α1 and β1 are found on astrocyte fibres surrounding select microvessels in the adult primate [26,46]. In addition, murine primary astrocytes in culture express integrins α1β1, α3β1, α5β1 and α6β1 [47,48]. Function-blocking studies show that these integrins are active adhesion receptors for laminin (α1β1, α3β1 and α6β1) and fibronectin (α5β1) [49]. In culture, astrocytes also express integrins αvβ5 and αvβ8, which, on a vitronectin substrate, promote astrocyte adhesion and migration respectively [49].

Astrocyte end-feet are complex structures that reside in close proximity to the abluminal surfaces of endothelial cells. Integrin α6β4 is expressed on the astrocyte end-feet.
in a small proportion of normal capillaries and non-capillary microvessels [26,46]. The reasons for this restricted expression are so far unknown. Laminin-5 is a matrix ligand for integrin α6β4, co-distributed in the cerebral microvascular basal lamina together with the major matrix constituents laminin-1, collagen type IV, and cellular fibronectin [46]. Hemidesmosomes, which anchor epithelial cells to the cuticular basement membrane, contain the integrin α6β4, and have been found in astrocyte end-feet at the vascular basal lamina interface of larger microvessels [50]. In short, integrin α6β4 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.

**Adhesion receptor expression during focal ischaemia**

Changes in microvessel integrin expression occur within 1–2 h in the ischaemic core, and are accompanied by detachment of astrocyte end-feet, local loss of permeability barrier integrity, oedema accumulation, and the appearance of markers of angiogenesis (Table 1) [24,25,36,51,52]. 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 approx. 60% of the baseline in the ischaemic core by 24 h after MCA:O (middle cerebral artery occlusion) [8]. But, within 2 h, there is significantly greater loss of perlecan than laminin within the basal lamina, indicating the greater sensitivity of this integrin β1 ligand to focal ischaemia [53]. These events correlate with the simultaneous generation of the MMPs (matrix proteinases) pro-MMP-2, pro-MMP-9 and their activators urokinase [uPA (urokinase-type plasminogen activator)], MT1 (membrane-type 1)-MMP, and MT3-MMP on microvessels (and neurons) within the regions of injury [54–56]. Cysteine proteinase activity, marked by the appearance of cathepsin L, corresponds to the loss of perlecan [53]. Importantly, loss of the major basal lamina proteins is directly associated with extravasation of erythrocytes and haemorrhagic transformation [57].

During focal ischaemia, the microvascular endothelium appears intact, while the astrocyte end-feet are displaced from the intact basal lamina. Rapid alterations in adhesion receptor expression occur on both sides of the vascular matrix.

By 2 h following MCA:O, β1-integrin expression by the endothelium is lost in 69 ± 7% of microvessels within the ischaemic core [36]. This loss is sustained and does not recover despite restitution of flow [36]. The mechanisms by which ischaemia diminishes microvessel β1-expression are not yet known. However, within the ischaemic core, down-regulation of microvessel-associated integrin β1 gene appears heterogeneous, but in a consistent manner: confluent regions of increased β1 transcription on microvessels surround sub-regions with decreased β1 expression early following MCA:O [36]. The up-regulation of β1 in the boundary between the ischaemic core and the immediately affected peripheral zone, and around subregions lacking expression, is consistent with the notion that the ‘ischaemic penumbra’ is initially interspersed among relatively unaffected tissue within the core [36]. In time, all β1 gene expression ceases as the injured subregions merge.

Within the ischaemic core, the subunits α1, α3 and α6 are significantly decreased in parallel with the β1 subunit [26], suggesting that all endothelial-cell β1-integrin complexes are affected by ischaemia. Endothelial-cell α1β1 expression appears sustained on microvessels which retain laminin within the basal lamina; the loss of this integrin generally precedes the loss of laminin [36]. But there is no evidence of endothelial-cell detachment early following MCA:O.

Simultaneously, focal ischaemia stimulates a consistent and significant early expression of the integrin αvβ3 on activated non-capillary cerebral microvessels [51,58]. Tissue culture studies have shown that integrin αvβ3 is expressed by cerebral endothelial cells, but not astrocytes [49]. While αvβ3 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. The expressions of integrin αvβ3 and VEGF (vascular endothelial growth factor) on activated microvessels are co-ordinated and directly related as early as 2 h following MCA:O [51]. This significant relationship is independent of time, reflecting the heterogeneity of the evolving injury in the striatum [51]. Subunit αv transcription is up-regulated along with VEGF in affected microvessels within the ischaemic core [51]. Also, a highly significant relationship between microvessel αvβ3 expression and fibrin deposition (one of its ligands) within the microvasculature has been observed [51]. These changes indicate the activation of the endothelium in the acute moments of ischaemia when matrix receptor expression is being lost.

Across the basal lamina, alteration in the interactions of astroglia with the intervening matrix has been suggested by loss of matrix intensity and detachment of these cells [8,34,52,53]. Following the onset of focal ischaemia, integrin α6β4 is rapidly lost from the astrocyte end-feet of select microvessels [46]. This corresponds to the separation of astrocytes from the vascular matrix early following MCA:O, and to the cell swelling and loss in cytoplasmic density that accompanies astrocyte separation. These changes in ultrastructure and integrin α6β4 expression also imply fundamental alterations in the end-foot apparatus. However, careful observation of the distribution of integrin α6β4 or the subunit β4 in normal striatal microvessels suggests (i) that the expression of this adhesion receptor on glial end-feet is highly specialized, and (ii) the adhesion of end-feet to the abluminal matrix must involve another group or family of receptors.

Finally, blockade of integrin–matrix interactions by the exogenous application of integrin-specific inhibitors has been attempted successfully in experimental systems with leukocyte β2-integrins [59] and with the platelet-fibrin(ogen) receptor αIIbβ3 [60,61]. Concerns regarding the safety of the use of pan-β3 inhibitors have been raised. Experiments involving inhibitors of the cell adhesion receptor–matrix
interactions within the cerebral microvasculature or extrava- 
sular targets have been few.

**Alteration in the permeability barrier following focal ischaemia**

The dystroglycan complex is clearly expressed on the cere-
brovasculature of both primate and murine brain tissues. Interestingly, dystroglycan, an established receptor for laminin and perlecan, shows a different pattern of expression from that of the α6β4-integrin. While both are located on the end-feet of glial processes that contact the vascular basal lamina, dual-colour immunofluorescent studies have shown that dystroglycan is expressed on blood vessels of all diameters, and on blood vessels throughout white and grey matter. In contrast, the integrin α6β4 shows a more restricted distribution pattern. Analysis of cell-surface expression of dystroglycan on cells in culture reveals that the dystroglycan complex is expressed at higher levels on astrocytes than endothelial cells. Studies examining the impact of ischaemia and hypoxia on astrocyte dystroglycan expression have shown comparable effects. Ongoing experiments suggest the involvement of several different protein systems as possible molecular mechanisms responsible for this distinct regulation. Furthermore, an impact on the integrity of the blood–brain barrier has been suggested.

**Adhesion receptors and the permeability barrier**

Matrix adhesion by both endothelial cells and astrocytes appears central to the maintenance of capillary integrity in the CNS. Select integrins and the dystroglycan complex are expressed at high levels specifically at the blood–brain barrier, and during focal cerebral ischaemia, decreases in their expressions coincide with breakdown of the permeability barrier. Studies with murine transgenic preparations show that the absence of specific integrins is associated with changes in permeability barrier, and during focal cerebral ischaemia, decreases in their expressions coincide with breakdown of the permeability barrier. The preparation of this paper was supported in part by grants RO1 NS026945, R37 NS038710 and RO1 NS053716 from the National Institutes of Health (Bethesda, MD, U.S.A.). We are indebted to the expertise and personal contributions of Greta Berg to this paper.

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**References**


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