Functions of lipid raft membrane microdomains at the blood–brain barrier

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Received: 20 March 2009 / Revised: 29 April 2009 / Accepted: 4 May 2009 / Published online: 30 May 2009
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Abstract The blood–brain barrier (BBB) is a highly specialized structural and functional component of the central nervous system that separates the circulating blood from the brain and spinal cord parenchyma. Brain endothelial cells (BECs) that primarily constitute the BBB are tightly interconnected by multiprotein complexes, the adherens junctions and the tight junctions, thereby creating a highly restrictive cellular barrier. Lipid-enriched membrane microdomain compartmentalization is an inherent property of BECs and allows for the apicobasal polarity of brain endothelium, temporal and spatial coordination of cell signaling events, and actin remodeling. In this manuscript, we review the role of membrane microdomains, in particular lipid rafts, in the BBB under physiological

conditions and during leukocyte transmigration/diapedesis. Furthermore, we propose a classification of endothelial membrane microdomains based on their function, or at least on the function ascribed to the molecules included in such heterogeneous rafts: (1) rafts associated with interendothelial junctions and adhesion of BECs to basal lamina (*scaffolding rafts*); (2) rafts involved in immune cell adhesion and migration across brain endothelium (*adhesion rafts*); (3) rafts associated with transendothelial transport of nutrients and ions (*transporter rafts*).

Keywords Multiple sclerosis · Leukocyte transmigration · Neuroinflammation · Tight junction · Adherens junction · Cell adhesion molecule · Integrin

Electronic supplementary material The online version of this article (doi:10.1007/s00109-009-0488-6) contains supplementary material, which is available to authorized users.

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Introduction

The term blood–brain barrier (BBB) refers to the structurally and functionally unique microvasculature of the central nervous system (CNS) parenchyma. While the initial description of the BBB defined its physical barrier characteristics by its ability to restrict diffusion of water-soluble acidic dyes from the blood to the CNS parenchyma and vice versa [1–3], contemporary morphological definition(s) of the BBB have refined this concept and allowed for a physical extension of the BBB from the capillary beds to microvascular structures of the postcapillary venules. This morphological definition of the BBB arises from the combination of functional, cellular, and molecular studies which demonstrate that CNS microvessels, whether capillaries or postcapillary venules, are structurally and functionally distinct from microvessels in other organs. These differences include (1) the presence of vessel-associated pericytes, (2) two dense and compact basement membranes, (3) astrocytic foot processes ensheathing CNS vascular endothelium, and (4) the unique molecular composition and complexity of CNS microvessel tight junctions.

The BBB controls the diffusion of ions and toxic molecules into the CNS [4], while providing nutrients through expression of BBB-specific transporters, such as glucose transporter 1, sodium/glucose cotransporters, and transferrin receptor [4]. Intercellular junctional complexes between brain endothelial cells (BECs), namely tight junctions (TJs) and adherens junctions (AJ), provide endothelial polarity in addition to high transendothelial electrical resistance (TEER) and restrictive permeability [5]. Pericytes and perivascular astrocytes, through complex communications and signaling events with BECs, induce BBB properties and promote TJ maintenance, specialized transporter expression, and immune quiescence of BECs [4, 6–13]. In the current review, we make use of the term BBB to include molecular and functional events, which occur at the level of the highly specialized capillaries and of postcapillary venules of the CNS.

Lipid rafts are dynamic plasma membrane microdomains associated with membrane compartmentalization, actin rearrangements, and plasma membrane receptor-mediated signaling events [14–17]. The first experimental line of evidence for biological lipid rafts came from observations made using epithelial cells in which glycosphingolipids trafficked to the apical membrane of polarized epithelial monolayers [18, 19]. Subsequent studies demonstrated that membrane clusters of glycolipids were insoluble in detergents at 4°C, forming detergent-resistant membranes (DRMs). These observations, and others, have led to the *lipid raft hypothesis*, which states that lipid (or membrane) rafts, structurally defined as 10–200-nm structures, are

heterogeneous and highly dynamic biological membrane microdomains enriched in cholesterol and sphingolipids [14, 16]. A wide variety of proteins are known to be targeted to lipid rafts and raft microdomains have thus been associated with a wide range of biological processes, including endocytosis, signal transduction [20, 21], apoptosis, cell polarization [22, 23], adhesion, migration, synaptic transmission, and cytoskeleton tethering [15]. Although lipid rafts have been extensively studied in T cell activation and migration [20, 22], junctional proteins, adhesion molecules, and transporters can be physically located into lipid raft membrane microdomains of human and mouse BECs, and their expression and localization are regulated by inflammatory and glial-derived factors. In this manuscript, we will review the role of raft microdomains in BBB maintenance and during leukocyte transmigration processes.

Evidence for functionally distinct membrane rafts

The plasma membrane of eukaryotic cells is composed of a complex mixture of membrane proteins and lipid molecules, including glycerophospholipids, sterols, and sphingolipids. Glycerophospholipids tend to adopt a mobile loosely packed fluid phase while sterols and sphingolipids possess distinct biophysical properties and readily group tightly together in a more ordered state. The differential lipid packing abilities lead to phase separation and to the organization of sphingolipid- and cholesterol-rich membrane microdomains, commonly referred to as *rafts* [14, 16, 18, 24].

Experimental studies have demonstrated that membrane clusters of glycosphingolipids and sterols were insoluble in detergents at 4°C and form DRMs that can be isolated by ultracentrifugation in a sucrose gradient. These observations have led to the *lipid raft hypothesis* that defines lipid rafts as heterogeneous nanostructures enriched in cholesterol and sphingolipids [14, 16]. While most of these lipid raft isolation protocols [25] have been performed using Triton X (TX)-100 at 4°C [25, 26], many other detergents have also been used to prepare DRMs, including Lubrol [27], Brij-58, Brij-96, Brij-98 [28], NP40, CHAPS, and octylglucoside [25, 29]. Nondetergent fractionation methods have also been used to isolate membrane rafts and likely reproduce more accurately the *in vivo* protein oligomerizations that occur in microdomains [30, 31]. As a result, the lipid, cholesterol, and protein compositions within DRMs can vary between raft preparations, giving rise to biophysically diverse rafts. However, the heterogeneity of the lipid rafts, in addition to being an outcome of detergent selectivity, may also represent the underlying diversity of lipid and protein compositions. This could in

turn reflect the intrinsically different functions attributed to membrane rafts.

A wide variety of proteins are targeted to lipid rafts, notably, but not exclusively, via fatty acylation (myristate and palmitate modifications) and glycosylphosphatidylinositol (GPI) anchors. Small rafts may be stabilized to form larger platforms through protein–protein or protein–lipid interactions. The raft microdomains have thus been associated with a wide range of biological processes, including endocytosis, signal transduction [21], apoptosis, adhesion, migration, synaptic transmission, and cytoskeleton tethering [15]. In addition, the existence of distinct lipid constituents within membrane microdomains is important for cellular polarization [22]. Apically, the membrane is enriched in glycosphingolipids and sphingomyelin, favoring the targeting of GPI-anchored proteins. Conversely, proteins important for contacting neighboring cells and the underlying connective tissue are confined to the basolateral membrane, suggesting the presence, within individual cells, of functionally different membrane domains [32]. Segregation of functionally diverse microdomains is an attractive notion that would allow the clustering of molecules serving similar purposes together in order to optimize efficient intracellular signaling. These observations also suggest that membrane rafts have an active role in asymmetric redistribution of membrane proteins during cell migration. Compartmentalized lipid rafts have been described in leukocytes, and most research has focused on membrane raft function during lymphocyte migration [33, 34]. In endothelial cells (ECs), lipid membrane rafts also exist but their role and function are still not well defined [35].

A specialized subset of rafts known as caveolae, defined as small cave-like surface invaginations of 50–100 nm in diameter, has been shown to mediate vesicular transport and sophisticated cell signaling. Caveolin-1, the main protein component of these structures, functions as a scaffolding protein and as a potential cholesterol sensor, regulating raft polymerization and lipid trafficking [36, 37]. Unlike lipid rafts, caveolae are not present in all cell types but are found abundantly in ECs and aid in regulating numerous endothelial functions such as transcytosis, vascular permeability, angiogenesis and can serve as docking sites for glycolipids and GPI-linked proteins, as well as for various receptors and signaling molecules [38]. In addition, due to their similar biophysical properties, caveolae represent a subset of the DRM population and can be further purified using magnetic beads coupled to caveolin-1 antibodies [39].

Elegant studies by Barreiro et al. have shown that tetraspanin-enriched membrane microdomains, different from lipid rafts, may be crucial for EC function by acting as adhesion and signaling structures [40]. Tetraspanin platforms are formed by external homophilic and hetero-

philic interactions and intracellular associations with signaling proteins and adhesion molecules [41, 42]. The tetraspanins are a group of membrane-spanning molecules and are reported to form an integral part of the leukocyte docking structure or transmigration cup [40, 43]. CD9 (tetraspanins) is proposed to form an integral web linking cell adhesion molecules (CAMs) such as intercellular CAM-1 (ICAM-1) to vascular CAM-1 (VCAM-1) via its extracellular domain and help organize a plasma membrane signaling platform. Even though tetraspanins have been associated with leukocyte–EC interactions, the exact role(s) and function(s) of tetraspanins in ECs in health and disease remain to be established.

Raft microdomains and the BBB

The role of membrane microdomains in ECs has only recently been recognized as an important part of many vascular processes. Isolation of lipid rafts from human primary cultures of BECs has been performed using different techniques and detergents [8, 31]. Figure 1 shows different raft isolation preparations using four distinct mild detergents and illustrates variability with regard to the concentration of cholesterol, caveolin-1, and GM-1, all markers used to identify rafts. By using these protocols, BEC-derived DRMs enriched in caveolin-1 also incorporate TJ proteins, integrins, specific transporters, and CAMs. We thus propose a classification of BEC lipid rafts based on the function of molecules found in these membrane compartments: (1) rafts associated with interendothelial TJ formation and EC adhesion to the basal lamina (*scaffolding rafts*), (2) rafts involved in immune cell adhesion and recruitment across EC barriers (*adhesion rafts*), and (3) rafts associated with transendothelial transport activity (*transporter rafts*).

1. Scaffolding rafts

TJs consist of at least three known types of transmembrane proteins, occludin, claudins, and the junctional adhesion molecules (JAMs) [4, 5, 44, 45]. Overall, the expression levels of TJ proteins have been found to decrease in many neurological disorders that are characterized by loss of BBB permeability integrity, such as in multiple sclerosis (MS) [8, 46–48]. AJs of BECs are primarily composed of vascular endothelial cadherin (VE-cadherin), which interact with intracellular proteins, including the different catenin isoforms (α -, β -, γ -catenin) and ZO-1 to create the association with the actin cytoskeleton [49–54].

In order to maintain a stable and tight functional barrier, scaffolding rafts serve to regulate integrin-mediated attachment to the basal lamina, as well as TJ- and AJ-mediated

Fig. 1 Use of different detergents to isolate cholesterol-enriched microdomains from human primary cultures of BECs. Four detergents were used to optimize cholesterol-enriched DRM isolation from primary cultures of human BECs. Following treatment of the cells with the detergent, membrane microdomains were separated by sucrose-density centrifugation, as described in Wosik et al. [8] and Cayrol et al. [99]. Cholesterol and phospholipid concentrations, as well as GM-1, caveolin-1, and CD59, were used to identify raft fractions by Western blot. JAM-1 is shown as an example of a tight junction protein expressed in the raft fraction and its modulation according to the detergent used. A detailed protocol of raft preparation is available in the “[Supplementary material](#)” section

cell–cell association [55, 56]. Both AJs and TJs are important for the regulation of paracellular permeability of ions, solutes, and nutrients and both junctional complexes localize, at least in part to membrane rafts. Nusrat et al. first proposed that TJ molecules of epithelial cells were enriched in lipid raft membrane microdomains. By using the T84 intestinal epithelial cell line and TX-100 raft isolation procedures, occludin and ZO-1 were found to be significantly increased in the insoluble fractions compared to the non-raft-soluble fractions [57]. This functional membrane raft idea was adopted by others and recent publications confirmed the close association of membrane rafts with various TJ and AJ proteins in both endothelial and epithelial cells [58, 59]. Figure 2a shows examples of TJ proteins found in DRMs of human primary cultures of BECs, isolated using Brij-58 and sucrose gradient centrifugation.

Distribution of TJ proteins to lipid rafts can occur via specific posttranslational fatty acid modifications. Inhibition of palmitoylation, required for optimal claudin function, inhibits claudin enrichment into DRM domains and leads to a decrease in TJ barrier function [60]. Using a novel detergent-free OptiPrep density-gradient method to fractionate rat cerebral microvessels, McCaffrey and coworkers observed TJ proteins in isolated raft fractions and found that the molecular weights of claudin-5, ZO-1, and occludin contained in rafts were twice as large as the predicted weights. This suggests that raft microdomains could be an environment in which TJ oligomerization occurs [30, 31]. Other studies show that alterations in caveolin-1 expression induces alterations of the TJ and AJ complexes [36, 61] and caveolin-1 knockout mice suffer from microvascular hyperpermeability [62]. Song and colleagues [36] observed that caveolin-1 knockdown in BECs induced a shift in occludin, VE-cadherin, and β -catenin subcellular distribution from the DRM to the soluble fraction along with an increase in endothelial permeability. Taken together, these data demonstrate that specialized rafts contribute to barrier permeability through clustering of TJ and AJ proteins.

Several chemicals, described to affect cholesterol synthesis or availability, are known to modulate raft integrity. Modulation of cell membrane cholesterol by treating cells with the cholesterol chelator methyl- β -cyclodextrin (M β CD) induces an initial rise and subsequent fall in TEER measures, associated with actin redistribution and reduced membrane expression of occludin and ZO-1 [63, 64]. Moreover, it has been demonstrated that M β CD treatment leads to specific displacement of occludin, claudin-3, claudin-4, claudin-7, and JAM-A out of the Lubrol WX-resistant lipid rafts and coincides with a decrease in TJ integrity [65]. Nusrat et al. reported that cholesterol-lowering agents, such as HMG-CoA reductase inhibitors (i.e., statins), induced the mobilization of occludin outside of the raft compartment in epithelial cells [57]. In the human *in vitro* system, however, statins were shown to decrease BBB permeability without altering subcellular localization of occludin, JAM-A, VE-cadherin, ZO-1, or ZO-2 [66]. This suggests that protein localization into rafts is not uniquely regulated by cholesterol concentration and that additional effects of statins, unrelated to their cholesterol-lowering effects might account for these discrepancies. In that sense, we and others have shown that the barrier-promoting effect of statins was dependent on their induction of geranylation and independent of the cholesterol concentration [66, 67].

TJ enrichment within membrane rafts is positively influenced by astrocyte-secreted angiotensin metabolites and is negatively regulated by proinflammatory cytokines [8]. Similarly, additional proteins secreted by astrocytes can

Fig. 2 Representative Western blots of isolated lipid rafts probed for scaffolding, TJ proteins, and adhesion molecules. Lipid rafts were generated with treatment of primary cultures of human BECs with Brij 58 detergent followed by sucrose gradient centrifugation, as described in Wosik et al. [8] Representative raft fraction (R) and detergent-soluble fraction (S) were used to assess the presence of TJ, integrins, and adhesion molecules. The TJ proteins ZO-1, occludin, and JAM-1

(a) are present in the lipid raft fraction, in addition to caveolin-1. b Integrins α_5 and β_1 are readily detected in BEC lipid rafts. Integrin α_v is seen at lower levels and integrin β_3 does not seem to be associated with lipid raft under physiological conditions. c The cell adhesion molecules ICAM-1, ALCAM, and MCAM can be detected in lipid raft fractions of BECs under normal culture conditions

modulate the BEC–TJ microdomains, such as the chemokine CCL2 acting via its receptor CCR2 [68]. MCP-1 was reported to induce changes in caveolin-1 expression and to impact on ZO-1 and occludin localization in membrane microdomains, suggesting that, during inflammatory conditions, BBB destabilization can occur through inflammatory cytokine-induced reorganization of TJ proteins on the BEC surface [69].

The endothelial basement membrane and glia limitans formed by BECs and perivascular astrocyte end feet are composed of several extracellular matrix (ECM) components, including fibronectin, different laminin isoforms, collagen type IV, and heparan sulfate proteoglycans [11, 55, 70, 71]. Its dense and compact nature provides the ability to function as a substrate for soluble protein immobilization and cell attachment. The ECM is also an important obstacle to blood cell entry into the CNS parenchyma and is thought to be a crucial structure for cellular differentiation and gene expression induction. BECs constitutively express a number of integrins important for adhesion to the endothelial basal lamina, such as $\alpha_1\beta_1$, $\alpha_3\beta_1$, and $\alpha_6\beta_1$. There are several reports associating integrins with caveolae and lipid rafts [72]. Membrane rafts in ECs are known to be enriched in $\alpha_5\beta_1$ integrin, which binds to ECM components and mediates EC attachment to the basal lamina [56]. In that respect, although the significance of lipid raft-associated integrins remains to be established, our data presented in Fig. 2b suggest that Brij-58-derived lipid rafts from human BECs also contain integrin proteins, such as α_5 , α_v , and β_1 but not β_3 integrins. This confirms that distinct BEC membrane rafts are involved in the clustering of TJ, AJ, and specific integrins, which collectively mediate attachment of ECs to adjacent cells and to the vessel wall.

2. Adhesion rafts

Under physiological conditions, only low number of immune cells continuously cross the BBB and enter the CNS perivascular space in a process called immune surveillance. This process is highly regulated in both leukocytes and ECs and lipid rafts have been associated with all the steps of leukocyte recruitment across the endothelium [73, 74].

Leukocyte rolling is dependent on selectins and their respective carbohydrate ligands or on α_4 -integrins, at least in non-CNS organs. Selectin association with lipid rafts is important for selectin-dependent signaling [75, 76] and thus lipid rafts can influence selectin-dependent leukocyte–EC interactions. Although the exact role of endothelial selectin-dependent outside-in and inside-out intracellular signaling events can influence the migration of some immune cells in peripheral organs, there is still controversy on its role in the CNS [77–81].

Lymphocyte and myeloid cell diapedesis across CNS endothelium involves prototypic BBB CAMs such as ICAM-1 and ICAM-2 (for lymphocytes [82, 83]) and VCAM-1 (for myeloid cells) [84–86] which are expressed on the cell surface and/or at intercellular junctions and can associate with membrane microdomains (Fig. 2c) [87–89]. PECAM-1 is also known to act on leukocyte migration but has rather been documented to halt leukocyte emigration, as PECAM-1 knockout animals have enhanced immune cell infiltration into the CNS [90]. ICAM-1 and PECAM-1 are enriched in lipid rafts and signal through lipid raft platforms provided by membrane rafts [58, 91, 92]. The association of CAMs with cholesterol-enriched membrane microdomains is important for optimal signal transduction events during

Table 1 Membrane raft microdomain-associated transporters identified by proteomic analysis

Transport system	Lipid raft-associated transporter
Anion/cation transporters	<i>VDCA1, VDCA3</i>
Glutamate receptor	<i>GRIN2B</i>
Non-voltage-gated sodium channel	<i>SCNN1A</i>
Voltage-dependant calcium channel	<i>CACNA2D1</i>
Calcium-transporting ATPase	<i>ATP2B4</i>
Potassium/chloride solute carrier family	<i>SLC12A6, SLC4A3, SLC35E1, SLC41A2, HCN2</i>
ABC transporters	<i>ABCA6, ABCB7, and ABCA1</i>
Carbohydrate transporters	<i>SLC2A6, SLC45A4, SLC2A14</i>
Lipid and fatty acid transporters	<i>ApoB, ABCA6, PITPNM2, ABCA1</i>
Glycosyl, acetyl, methyl, or nucleoside transferases	<i>GGT1A1, MGAT2, UGT2B11, B4GalNac-T3, THUMP2, MAT1A</i>

Human primary cultures of BECs were grown to confluence and lysed in Brij 58 detergent and cholesterol-enriched membrane microdomains were isolated by sucrose gradient flotation. The raft fraction was subjected to trypsin digestion, separated by liquid chromatography and analyzed by mass spectrometry, as described in Cayrol et al. [99]

both transendothelial and transjunctional migration. Accordingly, knockdown of lipid raft-associated protein, caveolin-1, in ECs was shown to inhibit transendothelial leukocyte migration [93]. Recent studies using peripheral-derived endothelium and epithelial cultures have identified novel molecules that coordinate leukocyte transmigration, including JAMs, CD44, CD47, CD73 [94], CD90 [95], CD137 [96], CD81 [97], CD99 [98], CD99L, and CD166/ALCAM [99]. Most of these adhesion molecules are

enriched in lipid rafts derived from primary cultures of human BECs [99].

During the final phase of transmigration, complex intracellular signaling events take place in both the leukocyte and the EC to prepare the cells for transmigration/diapedesis. These events are dependent on adhesion molecule signaling and include cytoskeleton remodeling, TJ and AJ disassembly and reassembly, and cell body retraction. Recent data from Greenwood and colleagues

Fig. 3 Hypothetical model representing the functionally and spatially different lipid membrane rafts found in BECs. Scaffolding rafts secure the BECs to their surrounding and include rafts associated with interendothelial cell junction proteins (tight junction and adherens junction) and rafts associated with extracellular matrix attachment proteins. Adhesion rafts include microdomains associated with adhesion molecules found on the BECs and include rafts that would be enriched in proteins mediating interaction with peripheral leukocytes. Not shown in this model are the transporter rafts

support the notion that VE-cadherin relocation in BECs during diapedesis is dependent on ICAM-1 signaling events mediated by possible membrane raft signaling platforms. ICAM-1 signaling led to VE-cadherin phosphorylation and allowed paracellular leukocyte migration [58]. As recently described, a key feature of transendothelial migration (also called emperipolesis) is the formation of an EC cup-like structure surrounding the migrating leukocytes [40, 43, 73, 74, 100–103]. According to Carman et al., endothelial membranes positive for caveolin-1 are associated with the transmigrating leukocyte in the transmigration cup, which is dependent on the presence of lipid rafts and their recruitment of CAMs [74, 100]. In this context, lipid rafts are believed to coordinate outside-in signaling in ECs and regulation of actin remodeling by lipid raft-associated small guanosine triphosphatases, such as Rho [104–106] and Rac [107, 108], myosin-dependent contractions [109], and junctional protein regulation [8, 110]. These transendothelial migration events are highly organized around CAM-mediated signaling within lipid raft structures and support the concept of functionally distinct endothelial *adhesion rafts*.

Recently, tetraspanin-enriched membrane microdomains have been suggested to be equally important in acting as adhesion and signaling structures for leukocyte transmigration. Tetraspanins are small proteins (CD9, CD81, and CD151) that can organize as tetraspanin web microdomains (or nanoplateforms) and associate with numerous integral membrane proteins (ICAM-1 and VCAM-1) to provide a scaffold for specific interactions and outside-in signaling induction [42]. The exact differences between lipid rafts and tetraspanin platforms and their contribution during leukocyte migration are under active investigation. Nevertheless, the importance of tetraspanins in leukocyte migration to the brain has been shown by CD81 antibody blockade which inhibits monocyte migration and reduces clinical signs in experimental autoimmune encephalomyelitis (EAE), a validated animal model for MS [97]. Taken together, there is ample evidence to support the concept that lipid rafts and/or tetraspanin platforms contribute to the highly regulated process of immune cell transmigration across BECs.

3. Transporter Rafts

Transport of nutrients to the CNS is regulated by the expression of specific transporters in BECs. Expression of active transport systems for glucose, amino acids, nucleosides, fatty acids, minerals, vitamins, peptides, proteins, and ions define the prototypic nature and function of the BBB. In our liquid chromatography–mass spectrometry analysis of rafts obtained from primary cultures of human BBB endothelium [99], several members of the transporter system were identified. Due to the expected high number

of false-negative data generated by mass spectrometry, this list can only be considered partial or incomplete but includes many different transporter systems (see Table 1). These findings indicate that rafts might also play an active role in trans-BBB trafficking of numerous lipid insoluble molecules.

Conclusion

The presence of DRMs or membrane raft microdomains at the level of BECs appears to play a critical role in BBB function under physiological conditions and during complex cellular interactions. We have presented distinct functions of membrane microdomains in BEC physiology and during immune cell transmigration. We further propose a classification of membrane raft microdomains based on their function in the cells studied. While the proposed classification is not exhaustive, it is useful to identify functionally similar microdomains in heterogeneous populations of lipid rafts (Fig. 3). At the level of the BBB, we define *scaffolding rafts* as rafts associated with interendothelial junctions and adhesion of BECs to basal lamina, *adhesion rafts* as rafts involved in immune cell adhesion to and migration across brain endothelium and *transporter rafts* as rafts involved in the transendothelial movements of solutes.

References

1. Reese TS, Karnovsky MJ (1967) Fine structural localization of a blood–brain barrier to exogenous peroxidase. *J Cell Biol* 1:207–217
2. Brightman MW, Reese TS (1969) Junctions between intimately apposed cell membranes in the vertebrate brain. *J Cell Biol* 3:648–677
3. Goldmann EE (1913) VitalFarbung am Zentralnervensystem. *Abh Preuss Akad Wissensch PhysMath K1*:1–60
4. Zlokovic BV (2008) The blood–brain barrier in health and chronic neurodegenerative disorders. *Neuron* 2:178–201
5. Paris L, Tonutti L, Vannini C, Bazzoni G (2008) Structural organization of the tight junctions. *Biochim Biophys Acta* 3:646–659
6. Bundgaard M, Abbott NJ (2008) All vertebrates started out with a glial blood–brain barrier 4–500 million years ago. *Glia* 7:699–708
7. Lee SW, Kim WJ, Choi YK, Song HS, Son MJ, Gelman IH, Kim YJ, Kim KW (2003) SSeCKS regulates angiogenesis and tight junction formation in blood–brain barrier. *Nat Med* 7:900–906
8. Wosik K, Cayrol R, Dodelet-Devillers A, Berthelet F, Bernard M, Moudjian R, Bouthillier A, Reudelhuber TL, Prat A (2007) Angiotensin II controls occludin function and is required for blood brain barrier maintenance: relevance to multiple sclerosis. *J Neurosci* 34:9032–9042
9. Prat A, Biernacki K, Wosik K, Antel JP (2001) Glial cell influence on the human blood–brain barrier. *Glia* 2:145–155
10. Pachter JS, de Vries HE, Fabry Z (2003) The blood–brain barrier and its role in immune privilege in the central nervous system. *J Neuropathol Exp Neurol* 6:593–604

11. van Horssen J, Brink BP, de Vries HE, van der Valk P, Be L (2007) The blood–brain barrier in cortical multiple sclerosis lesions. *J Neuropathol Exp Neurol* 4:321–328
12. Garberg P, Ball M, Borg N, Cecchelli R, Fenart L, Hurst RD, Lindmark T, Mabondzo A, Nilsson JE, Raub TJ, Stanimirovic D, Terasaki T, Oberg JO, Osterberg T (2005) In vitro models for the blood–brain barrier. *Toxicol In Vitro* 3:299–334
13. Armulik A, Abramsson A, Betsholtz C (2005) Endothelial/pericyte interactions. *Circ Res* 6:512–523
14. Jacobson K, Mouritsen OG, Anderson RG (2007) Lipid rafts: at a crossroad between cell biology and physics. *Nat Cell Biol* 1:7–14
15. Viola A, Gupta N (2007) Tether and trap: regulation of membrane-raft dynamics by actin-binding proteins. *Nat Rev Immunol* 11:889–896
16. Mishra S, Joshi PG (2007) Lipid raft heterogeneity: an enigma. *J Neurochem* 103:135–142
17. Foster LJ, Chan QW (2007) Lipid raft proteomics: more than just detergent-resistant membranes. *Subcell Biochem* 43:35–47
18. Munro S (2003) Lipid rafts: elusive or illusive? *Cell* 4:377–388
19. Simons K, Ikonen E (1997) Functional rafts in cell membranes. *Nature* 6633:569–572
20. Manes S, Viola A (2006) Lipid rafts in lymphocyte activation and migration. *Mol Membr Biol* 1:59–69
21. Becher A, McIlhinney RA (2005) Consequences of lipid raft association on G-protein-coupled receptor function. *Biochem Soc Symp* 72:151–164
22. Gomez-Mouton C, Abad JL, Mira E, Lacalle RA, Gallardo E, Jimenez-Baranda S, Illa I, Bernad A, Manes S, Martinez A (2001) Segregation of leading-edge and uropod components into specific lipid rafts during T cell polarization. *Proc Natl Acad Sci U S A* 17:9642–9647
23. Manes S, Mira E, Gomez-Mouton C, Lacalle RA, Keller P, Labrador JP, Martinez A (1999) Membrane raft microdomains mediate front-rear polarity in migrating cells. *EMBO J* 22:6211–6220
24. Shaw AS (2006) Lipid rafts: now you see them, now you don't. *Nat Immunol* 11:1139–1142
25. Chamberlain LH (2004) Detergents as tools for the purification and classification of lipid rafts. *FEBS Lett* 1–3:1–5
26. Chen X, Morris R, Lawrence MJ, Quinn PJ (2007) The isolation and structure of membrane lipid rafts from rat brain. *Biochimie* 2:192–196
27. Delaunay JL, Breton M, Trugnan G, Maurice M (2008) Differential solubilization of inner plasma membrane leaflet components by Lubrol WX and Triton X-100. *Biochim Biophys Acta* 1:105–112
28. Gil C, Cubi R, Blasi J, Aguilera J (2006) Synaptic proteins associate with a sub-set of lipid rafts when isolated from nerve endings at physiological temperature. *Biochem Biophys Res Commun* 4:1334–1342
29. Pike LJ, Han X, Chung KN, Gross RW (2002) Lipid rafts are enriched in arachidonic acid and plasmalogen ethanolamine and their composition is independent of caveolin-1 expression: a quantitative electrospray ionization/mass spectrometric analysis. *Biochemistry* 6:2075–2088
30. McCaffrey G, Seelbach MJ, Staats WD, Nametz N, Quigley C, Campos CR, Brooks TA, Davis TP (2008) Occludin oligomeric assembly at tight junctions of the blood–brain barrier is disrupted by peripheral inflammatory hyperalgesia. *J Neurochem* 106:2395–2409
31. McCaffrey G, Staats WD, Quigley CA, Nametz N, Seelbach MJ, Campos CR, Brooks TA, Egleton RD, Davis TP (2007) Tight junctions contain oligomeric protein assembly critical for maintaining blood–brain barrier integrity in vivo. *J Neurochem* 103:2540–2555
32. Schuck S, Simons K (2004) Polarized sorting in epithelial cells: raft clustering and the biogenesis of the apical membrane. *J Cell Sci Pt* 25:5955–5964
33. Nguyen DH, Giri B, Collins G, Taub DD (2005) Dynamic reorganization of chemokine receptors, cholesterol, lipid rafts, and adhesion molecules to sites of CD4 engagement. *Exp Cell Res* 2:559–569
34. Gomez-Mouton C, Lacalle RA, Mira E, Jimenez-Baranda S, Barber DF, Carrera AC, Martinez A, Manes S (2004) Dynamic redistribution of raft domains as an organizing platform for signaling during cell chemotaxis. *J Cell Biol* 5:759–768
35. Mestas J, Hughes CC (2001) Endothelial cell costimulation of T cell activation through CD58-CD2 interactions involves lipid raft aggregation. *J Immunol* 8:4378–4385
36. Song L, Ge S, Pachter JS (2006) Caveolin-1 regulates expression of junction-associated proteins in brain microvascular endothelial cells. *Blood* 109:1515–1523
37. Pohl J, Ring A, Ehehalt R, Schulze-Bergkamen H, Schad A, Verkade P, Stremmel W (2004) Long-chain fatty acid uptake into adipocytes depends on lipid raft function. *Biochemistry* 14:4179–4187
38. Sprenger RR, Fontijn RD, van Marle J, Pannekoek H, Horrevoets AJ (2006) Spatial segregation of transport and signalling functions between human endothelial caveolae and lipid raft proteomes. *Biochem J* 3:401–410
39. Tang VW (2006) Proteomic and bioinformatic analysis of epithelial tight junction reveals an unexpected cluster of synaptic molecules. *Biol Direct* 1:37
40. Barreiro O, Zamai M, Yanez-Mo M, Tejera E, Lopez-Romero P, Monk PN, Gratton E, Caiolfa VR, Sanchez-Madrid F (2008) Endothelial adhesion receptors are recruited to adherent leukocytes by inclusion in preformed tetraspanin nanoplateforms. *J Cell Biol* 3:527–542
41. Levy S, Shoham T (2005) Protein–protein interactions in the tetraspanin web. *Physiology* 20:218–224
42. Levy S, Shoham T (2005) The tetraspanin web modulates immune-signalling complexes. *Nat Rev Immunol* 2:136–148
43. Barreiro O, de la Fuente H, Mittelbrunn M, Sanchez-Madrid F (2007) Functional insights on the polarized redistribution of leukocyte integrins and their ligands during leukocyte migration and immune interactions. *Immunol Rev* 218:147–164
44. Forster C (2008) Tight junctions and the modulation of barrier function in disease. *Histochem Cell Biol* 1:55–70
45. Persidsky Y, Ramirez SH, Haorah J, Kanmogne GD (2006) Blood–brain barrier: structural components and function under physiologic and pathologic conditions. *J Neuroimmune Pharmacol* 3:223–236
46. Leech S, Kirk J, Plumb J, McQuaid S (2007) Persistent endothelial abnormalities and blood–brain barrier leak in primary and secondary progressive multiple sclerosis. *Neuropathol Appl Neurobiol* 1:86–98
47. Allen IV, McQuaid S, Mirakhor M, Nevin G (2001) Pathological abnormalities in the normal-appearing white matter in multiple sclerosis. *Neurol Sci* 2:141–144
48. Yang Y, Estrada EY, Thompson JF, Liu W, Rosenberg GA (2007) Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rat. *J Cereb Blood Flow Metab* 4:697–709
49. Pokutta S, Drees F, Yamada S, Nelson WJ, Weis WI (2008) Biochemical and structural analysis of alpha-catenin in cell–cell contacts. *Biochem Soc Trans Pt* 2:141–147
50. Weber C, Fraemohs L, Dejana E (2007) The role of junctional adhesion molecules in vascular inflammation. *Nat Rev Immunol* 6:467–477
51. Nelson WJ (2008) Regulation of cell–cell adhesion by the cadherin–catenin complex. *Biochem Soc Trans Pt* 2:149–155

52. Nyqvist D, Giampietro C, Dejana E (2008) Deciphering the functional role of endothelial junctions by using in vivo models. *EMBO Rep* 8:742–747
53. Dejana E, Orsenigo F, Lampugnani MG (2008) The role of adherens junctions and VE-cadherin in the control of vascular permeability. *J Cell Sci Pt* 13:2115–2122
54. Danese S, Dejana E, Fiocchi C (2007) Immune regulation by microvascular endothelial cells: directing innate and adaptive immunity, coagulation, and inflammation. *J Immunol* 10:6017–6022
55. del Zoppo GJ, Milner R, Mabuchi T, Hung S, Wang X, Koziol JA (2006) Vascular matrix adhesion and the blood–brain barrier. *Biochem Soc Trans Pt* 6:1261–1266
56. Del Pozo MA (2004) Integrin signaling and lipid rafts. *Cell Cycle* 6:725–728
57. Nusrat A, Parkos CA, Verkade P, Foley CS, Liang TW, Innis-Whitehouse W, Eastburn KK, Madara JL (2000) Tight junctions are membrane microdomains. *J Cell Sci* 113:1771–1781
58. Turowski P, Martinelli R, Crawford R, Wateridge D, Papageorgiou AP, Lampugnani MG, Gamp AC, Vestweber D, Adamson P, Dejana E, Greenwood J (2008) Phosphorylation of vascular endothelial cadherin controls lymphocyte emigration. *J Cell Sci Pt* 1:29–37
59. Itoh M, Nagafuchi A, Yonemura S, Kitani-Yasuda T, Tsukita S, Tsukita S (1993) The 220-kD protein colocalizing with cadherins in non-epithelial cells is identical to ZO-1, a tight junction-associated protein in epithelial cells: cDNA cloning and immunoelectron microscopy. *J Cell Biol* 3:491–502
60. Van Itallie CM, Gamblin TM, Carson JL, Anderson JM (2005) Palmitoylation of claudins is required for efficient tight-junction localization. *J Cell Sci Pt* 7:1427–1436
61. Nag S, Venugopalan R, Stewart DJ (2007) Increased caveolin-1 expression precedes decreased expression of occludin and claudin-5 during blood-brain barrier breakdown. *Acta Neuropathol* 5:459–469
62. Schubert W, Frank PG, Razani B, Park DS, Chow CW, Lisanti MP (2001) Caveolae-deficient endothelial cells show defects in the uptake and transport of albumin in vivo. *J Biol Chem* 52:48619–48622
63. Lee DB, Jamgotchian N, Allen SG, Abeles MB, Ward HJ (2008) A lipid–protein hybrid model for tight junction. *Am J Physiol Renal Physiol* 6:F1601–F1612
64. Lynch RD, Francis SA, McCarthy KM, Casas E, Thiele C, Schneeberger EE (2007) Cholesterol depletion alters detergent-specific solubility profiles of selected tight junction proteins and the phosphorylation of occludin. *Exp Cell Res* 12:2597–2610
65. Lambert D, O'Neill CA, Padfield PJ (2007) Methyl-beta-cyclodextrin increases permeability of Caco-2 cell monolayers by displacing specific claudins from cholesterol rich domains associated with tight junctions. *Cell Physiol Biochem* 5:495–506
66. Ifergan I, Wosik K, Cayrol R, Kebir H, Auger C, Bernard M, Bouthillier A, Moumdjian R, Duquette P, Prat A (2006) Statins reduce human blood–brain barrier permeability and restrict leukocyte migration: relevance to multiple sclerosis. *Ann Neurol* 1:45–55
67. Greenwood J, Mason JC (2007) Statins and the vascular endothelial inflammatory response. *Trends Immunol* 2:88–98
68. Dimitrijevic OB, Stamatovic SM, Keep RF, Andjelkovic AV (2006) Effects of the chemokine CCL2 on blood–brain barrier permeability during ischemia–reperfusion injury. *J Cereb Blood Flow Metab* 6:797–810
69. Song L, Pachter JS (2004) Monocyte chemoattractant protein-1 alters expression of tight junction-associated proteins in brain microvascular endothelial cells. *Microvasc Res* 1:78–89
70. van Horssen J, Bo L, Dijkstra CD, de Vries HE (2006) Extensive extracellular matrix depositions in active multiple sclerosis lesions. *Neurobiol Dis* 3:484–491
71. Sixt M, Engelhardt B, Pausch F, Hallmann R, Wendler O, Sorokin LM (2001) Endothelial cell laminin isoforms, laminins 8 and 10, play decisive roles in T cell recruitment across the blood–brain barrier in experimental autoimmune encephalomyelitis. *J Cell Biol* 5:933–946
72. Wary KK, Mariotti A, Zurzolo C, Giancotti FG (1998) A requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. *Cell* 5:625–634
73. Carman CV, Springer TA (2008) Trans-cellular migration: cell–cell contacts get intimate. *Curr Opin Cell Biol* 20:533–540
74. Carman CV, Springer TA (2004) A transmigratory cup in leukocyte diapedesis both through individual vascular endothelial cells and between them. *J Cell Biol* 2:377–388
75. Engelhardt B, Kempe B, Merfeld-Clauss S, Laschinger M, Furie B, Wild MK, Vestweber D (2005) P-selectin glycoprotein ligand 1 is not required for the development of experimental autoimmune encephalomyelitis in SJL and C57BL/6 mice. *J Immunol* 2:1267–1275
76. Kiely JM, Hu Y, Garcia-Cardena G, Gimbrone MA Jr (2003) Lipid raft localization of cell surface E-selectin is required for ligation-induced activation of phospholipase C gamma. *J Immunol* 6:3216–3224
77. Kerfoot SM, Kubes P (2002) Overlapping roles of P-selectin and alpha 4 integrin to recruit leukocytes to the central nervous system in experimental autoimmune encephalomyelitis. *J Immunol* 2:1000–1006
78. Kerfoot SM, Norman MU, Lapointe BM, Bonder CS, Zbytniuk L, Kubes P (2006) Reevaluation of P-selectin and alpha 4 integrin as targets for the treatment of experimental autoimmune encephalomyelitis. *J Immunol* 10:6225–6234
79. Doring A, Wild M, Vestweber D, Deutsch U, Engelhardt B (2007) E- and P-selectin are not required for the development of experimental autoimmune encephalomyelitis in C57BL/6 and SJL mice. *J Immunol* 12:8470–8479
80. Uboldi C, Doring A, Alt C, Estess P, Siegelman M, Engelhardt B (2008) L-Selectin-deficient SJL and C57BL/6 mice are not resistant to experimental autoimmune encephalomyelitis. *Eur J Immunol* 8:2156–2167
81. Piccio L, Rossi B, Scarpini E, Laudanna C, Giagulli C, Issekutz AC, Vestweber D, Butcher EC, Constantin G (2002) Molecular mechanisms involved in lymphocyte recruitment in inflamed brain microvessels: critical roles for P-selectin glycoprotein ligand-1 and heterotrimeric G(i)-linked receptors. *J Immunol* 4:1940–1949
82. Reiss Y, Hoch G, Deutsch U, Engelhardt B (1998) T cell interaction with ICAM-1-deficient endothelium in vitro: essential role for ICAM-1 and ICAM-2 in transendothelial migration of T cells. *Eur J Immunol* 10:3086–3099
83. Lyck R, Reiss Y, Gerwin N, Greenwood J, Adamson P, Engelhardt B (2003) T-cell interaction with ICAM-1/ICAM-2 double-deficient brain endothelium in vitro: the cytoplasmic tail of endothelial ICAM-1 is necessary for transendothelial migration of T cells. *Blood* 10:3675–3683
84. Floris S, Ruuls SR, Wierinckx A, van der Pol SM, Dopp E, van der Meide PH, Dijkstra CD, de Vries HE (2002) Interferon-beta directly influences monocyte infiltration into the central nervous system. *J Neuroimmunol* 1–2:69–79
85. Seguin R, Biernacki K, Rotondo RL, Prat A, Antel JP (2003) Regulation and functional effects of monocyte migration across human brain-derived endothelial cells. *J Neuropathol Exp Neurol* 4:412–419
86. Ifergan I, Kebir H, Bernard M, Wosik K, Dodelet-Devillers A, Cayrol R, Arbour N, Prat A (2008) The blood–brain barrier induces differentiation of migrating monocytes into Th17-polarizing dendritic cells. *Brain Pt* 3:785–799

87. Muller WA (2001) Migration of leukocytes across endothelial junctions: some concepts and controversies. *Microcirculation* 3:181–193
88. Springer TA (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 2:301–314
89. Ley K, Laudanna C, Cybulsky MI, Nourshargh S (2007) Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol* 9:678–689
90. Graesser D, Solowiej A, Bruckner M, Osterweil E, Juedes A, Davis S, Ruddle NH, Engelhardt B, Madri JA (2002) Altered vascular permeability and early onset of experimental autoimmune encephalomyelitis in PECAM-1-deficient mice. *J Clin Invest* 3:383–392
91. Amos C, Romero IA, Schultze C, Rousell J, Pearson JD, Greenwood J, Adamson P (2001) Cross-linking of brain endothelial intercellular adhesion molecule (ICAM)-1 induces association of ICAM-1 with detergent-insoluble cytoskeletal fraction. *Arterioscler Thromb Vasc Biol* 5:810–816
92. Muller WA, Weigl SA, Deng X, Phillips DM (1993) PECAM-1 is required for transendothelial migration of leukocytes. *J Exp Med* 2:449–460
93. Millan J, Hewlett L, Glyn M, Toomre D, Clark P, Ridley AJ (2006) Lymphocyte transcellular migration occurs through recruitment of endothelial ICAM-1 to caveola- and F-actin-rich domains. *Nat Cell Biol* 2:113–123
94. Mills JH, Thompson LF, Mueller C, Waickman AT, Jalkanen S, Niemela J, Airas L, Bynoe MS (2008) CD73 is required for efficient entry of lymphocytes into the central nervous system during experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* 27:9325–9330
95. Wetzel A, Chavakis T, Preissner KT, Sticherling M, Hausteil UF, Anderegg U, Saalbach A (2004) Human Thy-1 (CD90) on activated endothelial cells is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). *J Immunol* 6:3850–3859
96. Drenkard D, Becke FM, Langstein J, Spruss T, Kunz-Schughart LA, Tan TE, Lim YC, Schwarz H (2007) CD137 is expressed on blood vessel walls at sites of inflammation and enhances monocyte migratory activity. *FASEB J* 2:456–463
97. Dijkstra S, Kooij G, Verbeek R, van der Pol SM, Amor S, Geisert EE Jr, Dijkstra CD, van Noort JM, Vries HE (2008) Targeting the tetraspanin CD81 blocks monocyte transmigration and ameliorates EAE. *Neurobiol Dis* 3:413–421
98. Bixel G, Kloep S, Butz S, Petri B, Engelhardt B, Vestweber D (2004) Mouse CD99 participates in T-cell recruitment into inflamed skin. *Blood* 10:3205–3213
99. Cayrol R, Wosik K, Berard JL, Dodelet-Devillers A, Ifergan I, Kebir H, Haqqani AS, Kreymborg K, Krug S, Moumdjian R, Bouthillier A, Becher B, Arbour N, David S, Stanimirovic D, Prat A (2008) Activated leukocyte cell adhesion molecule promotes leukocyte trafficking into the central nervous system. *Nat Immunol* 2:137–145
100. Carman CV, Jun CD, Salas A, Springer TA (2003) Endothelial cells proactively form microvilli-like membrane projections upon intercellular adhesion molecule 1 engagement of leukocyte LFA-1. *J Immunol* 11:6135–6144
101. Barreiro O, Yanez-Mo M, Sala-Valdes M, Gutierrez-Lopez MD, Ovalle S, Higginbottom A, Monk PN, Cabanas C, Sanchez-Madrid F (2005) Endothelial tetraspanin microdomains regulate leukocyte firm adhesion during extravasation. *Blood* 7:2852–2861
102. Barreiro O, Vicente-Manzanares M, Urzainqui A, Yanez-Mo M, Sanchez-Madrid F (2004) Interactive protrusive structures during leukocyte adhesion and transendothelial migration. *Front Biosci* 9:1849–1863
103. Barreiro O, Yanez-Mo M, Serrador JM, Montoya MC, Vicente-Manzanares M, Tejedor R, Furthmayr H, Sanchez-Madrid F (2002) Dynamic interaction of VCAM-1 and ICAM-1 with moesin and ezrin in a novel endothelial docking structure for adherent leukocytes. *J Cell Biol* 7:1233–1245
104. Ridley AJ (2001) Rho proteins: linking signaling with membrane trafficking. *Traffic* 5:303–310
105. Honing H, Van Den Berg TK, van der Pol SM, Dijkstra CD, van der Kammen RA, Collard JG, de Vries HE (2004) RhoA activation promotes transendothelial migration of monocytes via ROCK. *J Leukoc Biol* 3:523–528
106. Stamatovic SM, Keep RF, Kunkel SL, Andjelkovic AV (2003) Potential role of MCP-1 in endothelial cell tight junction ‘opening’: signaling via Rho and Rho kinase. *J Cell Sci Pt* 22:4615–4628
107. Prieto-Sanchez RM, Berenjeno IM, Bustelo XR (2006) Involvement of the Rho/Rac family member RhoG in caveolar endocytosis. *Oncogene* 21:2961–2973
108. Fujitani M, Honda A, Hata K, Yamagishi S, Tohyama M, Yamashita T (2005) Biological activity of neurotrophins is dependent on recruitment of Rac1 to lipid rafts. *Biochem Biophys Res Commun* 1:150–154
109. Ishmael JE, Safic M, Amparan D, Vogel WK, Pham T, Marley K, Filtz TM, Maier CS (2007) Nonmuscle myosins II-B and Va are components of detergent-resistant membrane skeletons derived from mouse forebrain. *Brain Res* 1143:46–59
110. Li Q, Zhang Q, Zhang M, Wang C, Zhu Z, Li N, Li J (2008) Effect of n-3 polyunsaturated fatty acids on membrane microdomain localization of tight junction proteins in experimental colitis. *FEBS J* 3:411–420