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Blood Brain Barrier

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Abstract
This article introduces the special issue on "Blood–Brain Barrier and Epilepsy." We review briefly current understanding of the structure and function of the blood–brain barrier (BBB), including its development and normal physiology, and ways in which it can be affected in pathology. The BBB formed by the endothelium of cerebral blood vessels is one of three main barrier sites protecting the central nervous system (CNS). The barrier is not a rigid structure, but a dynamic interface with a range of interrelated functions, resulting from extremely effective tight junctions, transendothelial transport systems, enzymes, and regulation of leukocyte permeation, which thereby generates the physical, transport, enzymatic, and immune regulatory functions of the BBB. The brain endothelial cells are important components of a "modular" structure, the neurovascular unit (NVU), with several associated cell types and extracellular matrix components. Modern methods have helped in identifying a range of proteins involved in barrier structure and function, and recent studies have revealed important stages, cell types, and signaling pathways important in BBB development. There is a growing list of CNS pathologies showing BBB dysfunction, with strong evidence that this can play a major role in certain disease etiologies
Keywords- Blood–brain barrier, Brain diseases, Neurovascular unit

I. INTRODUCTION

The brain and spinal cord (central nervous system, CNS) are the control centers of the body, generating central programs and strategies, processing sensory input, regulating motor output, and coordinating many of the individual and concerted activities of tissues. Moreover, networks of CNS neurons use a combination of chemical and electrical signals, which involve precise ionic movements across their membranes. Hence, to work effectively it is crucial that the CNS maintains a stable internal microenvironment. The active "neural" cells of the CNS, including neurons, macroglia (astrocytes, oligodendrocytes), and microglia contribute to local "housekeeping" maintenance of their bathing medium, the interstitial (or extracellular) fluid (ISF, ECF), whereas cellular barriers at the interfaces between the CNS and the circulating blood act as key regulatory sites, by controlling molecular flux into and out of the CNS. Thus essential nutrients are delivered, waste products removed, and entry ofpotentially toxic or neuroactive agents and pathogens is severely restricted.

The brain is precious, and evolution has gone to great lengths to protect it from damage. The most obvious is our 7mm thick skull, but the brain is also surrounded by protective fluid (cerebrospinal – of the brain and spine) and a protective membrane called the meninges. Both provide further defence against physical injury.Another protective element is the blood–brain barrier. As the name suggests, this is a barrier between the brain's blood vessels (capillaries) and the cells and other components that make up brain tissue. Whereas the skull, meninges and cerebrospinal fluid protect against physical damage, the blood–brain barrier provides a defence against disease-causing pathogens and toxins that may be present in our blood. The brain is the only organ known to have its own security system, a network of blood vessels that allows the entry of essential nutrients while blocking other substances. Unfortunately, this barrier is so effective at protecting against the passage of foreign substances that it often prevents life-saving drugs from being able to repair the injured or diseased brain. New studies are guiding researchers toward creative ways to open this barrier and "trick" it into allowing medicinesto enter.

II. WHAT IS BLOOD BRAIN BARRIER?

Blood-Brain Barrier (BBB) is a selectively permeable membrane regulates the passage of a multitude of large and small molecules into the microenvironment of the neurons. It achieves this feat by with the aid of multiple cellular transport channels scattered along the membrane. These include:

- amino acid transporters
- glucose transporter 1 (GLUT1)
- nucleouside & nucleotide transporters
- monocarboxylate transporters (MCT1 and MCT2)
- ion transporters (Na+/K+-ATPase pumps) that facilitate the transport of essential molecules into

the brain.

In addition to facilitating the uptake of amino acids, the amino acid transporters may inadvertently transport undesirable heavy metals into the brain's immediate environment. Consequently, at high enough concentrations, this will result in neurotoxicity. GLUT1 and the MCT transporters carry glucose, and lactate and ketones, respectively.

III. STRUCTURE OF BLOOD BRAIN BARRIER

Although brain capillaries are morphologically similar to those found in other tissues, brain vessels are functionally bound to the other cells of the brain parenchyma. BBB consists of blood vessels built up by specialized endothelial cells (ECs), astrocytes, pericytes, and neuronal terminations.Astrocytes lay their end-feet over the continuous basal lamina and form a very restrictive barrier.Pericytes, a type of mesenchymal cell, occupy the perivascular space, between the capillary wall and astrocytes end- feet, except in the large vessels where smooth muscle cells replace them. Pericytes play a regulatory role in vasculature tone, stability, repair and angiogenesis,being also able to modulate astroglial function. Finally, neurons are also participating actively in this structure since neuronal terminations arrive to all cells forming the BBB.

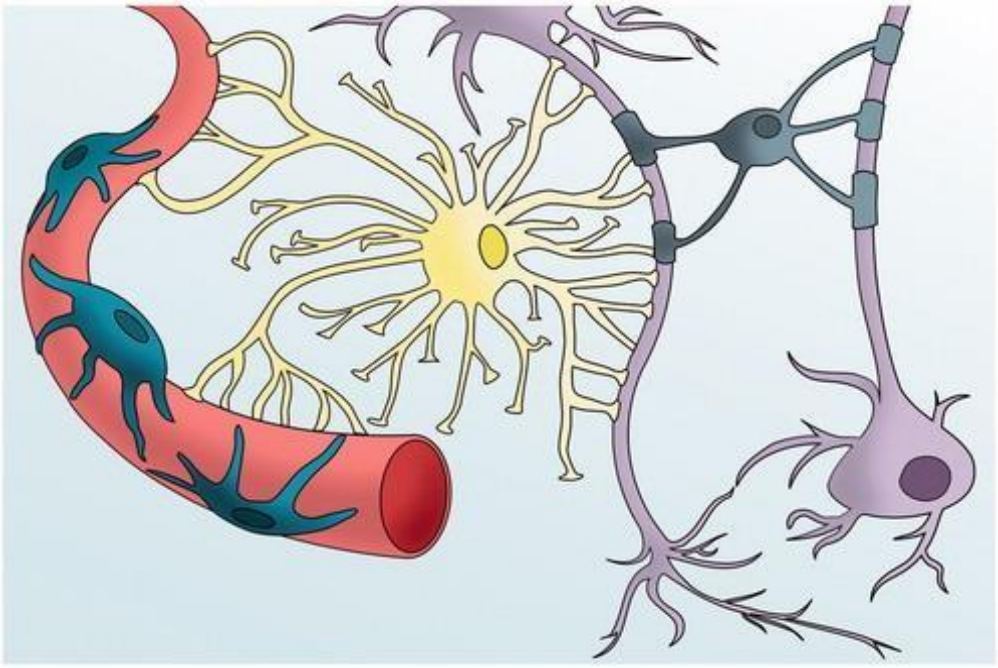

Fig 1. The BBB and the neurovascular unit. The blood brain barrier consist of a modified endothelium,which overexpresses tight junctions and Adherens junctions, surrounded by pericytes, astrocytical processes and neurons.

IV. CELLS OF BLOOD BRAIN BARRIER

Blood vessels are made up of two main cell types: ECs that form the walls of the blood vessels, and mural cells that sit on the ablumenal surface of the EC layer. The properties of the BBB are largely manifested within the ECs, but are induced and maintained by critical interactions with mural cells, immune cells, glial cells, and neural cells, which interact in the neurovascular unit

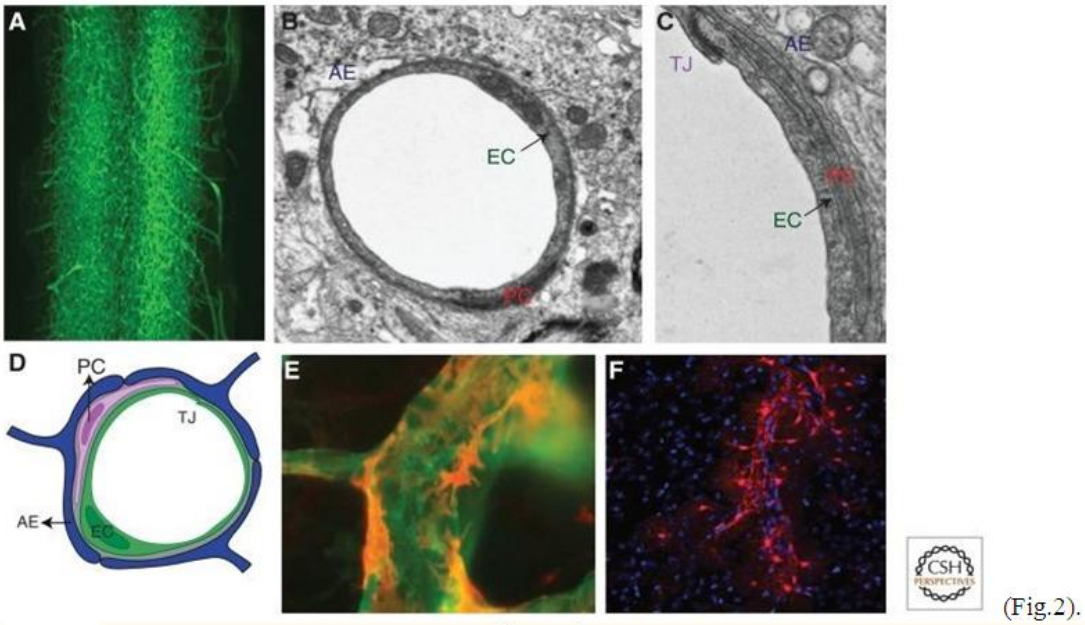

Figure 2.

(Fig.2).

Components of the BBB. (A) Vascular cast of a spinal cord showing density of the CNS vascular network. (B) Electron micrograph (EM) of a cross section of a CNS vessel depicting a relationship among endothelial cells (ECs), pericytes (PCs), and astrocytes. (C) Magnified EM of ECs depicting a relationship among ECs (with tight junctions [TJ]), PCs, basement membranes (BMs), and astrocyte endfeet (AE). (D) Schematic representation of the cell types within the neurovascular unit. (E) Immunofluorescence micrograph depicting relationship of PCs (red) with ECs (green). (F) Micrograph depicting relationship of astrocytes with blood vessels (unstained). Astrocytes extend processes that ensheath the blood vessels, such that the outline of the blood vessels can be visualized by the endfeet

4.1 Endothelial Cells

Endothelial cells (ECs) are mesodermally derived modified simple squamous epithelial cells that form the walls of blood vessels. The diameter of large arteries and veins can be made up of dozens of ECs, whereas the smallest capillary is formed by a single EC folding onto itself to form the lumen of the vessel. These CNS microvascular ECs are extremely thin cells that are 39% less thick than muscle ECs, with a distance of less than a quarter of a micron separating the lumenal from the parenchymal surface.

CNS ECs have unique properties compared with ECs in other tissues that allow them to tightly regulate the movement of ions, molecules, and cells between the blood and the brain. CNS ECs are held together

by tight junctions (TJs), which greatly limit the paracellular flux of solutes.CNS ECs undergo extremely low rates of transcytosis as compared with peripheral ECs, which greatly restricts the vesicle-mediated transcellular movement of solutes.This tight paracellular and transcellular barrier creates a polarized cell with distinct lumenal and ablumenal membrane compartments such that movement between the blood and the brain can be tightly controlled through regulated cellular transport properties.

There are two main categories of transporters expressed by CNS ECs. The first are efflux transporters, which are polarized to the lumenal surface and transport a wide variety of lipophilic molecules that could otherwise diffuse across the cell membrane, toward the blood. The second are highly specific nutrient transporters that facilitate the transport of specific nutrients across the BBB into the CNS, as well as removal of specific waste products from the CNS into the blood.CNS ECs contain higher amounts of mitochondria compared to other ECs, which is thought to be critical to generate ATP to drive the ion gradients critical for transport functions. CNS ECs also express an extremely low level of leukocyte adhesion molecules (LAMs), as compared with ECs in other tissues greatly limiting the amount of immune cells that enter the CNS.In addition, there is thought to be differential vascular metabolism in CNS ECs generating a barrier by altering the physical properties of molecules, which can change their reactivity, solubility, and transport properties. The combination of physical barrier properties (TJs, low transcytosis), molecular barrier properties (efflux transporters, specific metabolism, low LAMs), as well as specific transporters to deliver required nutrients, allows the ECs to tightly regulate CNS homeostasis.

A major question remains whether the BBB in different regions of the brain possess unique properties required for the function of the local neural circuitry. For instance, localized transport of specific nutrients could

be important for the development or functions of specific subclasses of neurons. Although most regions of the CNS are vascularized by capillaries that contain BBB properties, specific nuclei adjacent to the third and fourth ventricles, including the subfornical organ, area postrema, pineal gland, and median eminence, contain vessels that have a much greater passive permeability.The capillaries of these circumventricular organs are continuous fenestrated vessels, with a high permeability to solutes. This high permeability is important for the functions of these nuclei, which either sense blood solute concentrations or secrete molecules into the blood.

4.2 Mural Cells

Mural cells include vascular smooth muscle cells that surround the large vessels and pericytes, which incompletely cover the endothelial walls of the microvasculature. Pericytes (PCs) are cells that sit on the ablumenal surface of the microvascular endothelial tube, and are embedded in the vascular BM.A difficulty in studying PCs is the lack of a specific marker that is expressed uniquely by PCs, and, thus, these cells are often confused with other cells that sit in the perivascular space.Currently, the most widely accepted molecular identifier of CNS PCs is positive reactivity to both PDGFR-β and NG2; but other markers, including Anpep (CD13), desmin, Rgs5, Abcc9, Kcnj8, Dlk, and Zic1, have all been used to identify PCs, with none being perfect identifiers of this cell type.Pericytes extend long cellular processes along the ablumenal surface of the endothelium that can often span several EC bodies. These cells contain contractile proteins, and have the ability to contract to control the diameter of the capillary.Although these cells line the endothelial tube, most of the cell body and processes do not touch the endothelium, but are separated by the BM they are embedded within. The processes do form cellular adhesions with the endothelium at discrete points, described as peg-and-socket junctions, and are mediated by the adhesion molecule N-cadherin.In addition, other pericyte-endothelial cellular adhesions have been identified including adhesion plaques, gap junctions, and tight junctions.

CNS PCs have been shown to have unique properties compared to PCs in other tissues. CNS PCs are derived from the neural crest, in contrast with PCs in many peripheral tissues, which are derived from the mesoderm.In addition, CNS microvasculatures have the highest CNS PCs coverage of any tissue, with an endothelial:pericyte ratio estimated between 1:1 and 3:1, whereas the muscle has a ratio of 100:1.Pericytes play important roles in regulating angiogenesis, deposition of extracellular matrix, wound healing, regulating immune cell infiltration, and regulation of blood flow in response to neural activity, and reports suggest that they also can be multipotent stem cells of the CNS.In addition, these cells have been shown to be important for regulating the formation of the BBB during development, as well as maintaining its function in adulthood and aging.One of the major questions in pericyte biology is whether there are different subsets of PCs that may have different functions. Owing to the lack of defining markers, it remains unclear whether all of the different functions attributed to PCs are performed by all of the same cells, by different subsets of PCs, or even by nonpericyte cells that sit adjacent to the vasculature. The identification of new PC-specific markers, as well as the potential identification of markers of subsets of PCs will aid in clearing up these issues.

4.3 Basement Membrane

The vascular tube is surrounded by two BMs, the inner vascular BM and the outer parenchymal BM, also called the vascular glia limitans perivascularis.The vascular BM is an extracellular matrix secreted by the ECs and PCs, whereas the parenchymal BM is primarily secreted by astrocytic processes that extend toward the vasculature. These BMs are comprised of different secreted molecules including type IV collagens, laminin, nidogen, heparin sulfate proteoglycans, and other glycoproteins. The vascular and parenchymal BMs have a different composition, for instance, the former is made up of laminins α4 and α5, whereas the latter contains laminins α1 and α2. These BMs provide an anchor for many signaling processes at the vasculature, but also provide an additional barrier for molecules and cells to cross before accessing the neural tissue. Disruption of these BMs by matrix matalloproteinases is an important component of BBB dysfunction and leukocyte infiltration that is observed in many different neurological disorders.

4.4 Astrocytes

Astrocytes are a major glial cell type, which extend polarized cellular processes that ensheath either neuronal processes or blood vessels.The endfeet of the basal process almost completely ensheath the vascular tube, and contain a discrete array of proteins including dystroclycan, dystrophin, and aquaporin 4. The dysroglycan–dystrophin complex is important to link the endfeet cytoskeleton to the BM by binding agrin.This linkage coordinates aquaporin 4 into orthogonal arrays of particles, which is critical for regulating water homesostasis in the CNS. Astrocytes provide a cellular link between the neuronal circuitry and blood vessels. This neurovascular coupling enables astrocytes to relay signals that regulate blood flow in response to neuronal activity.This includes regulating the contraction/dilation of vascular smooth muscle cells surrounding arterioles as well as PCs surrounding capillaries. Astrocytes have been identified as important mediators of BBB formation and function because of the ability of purified astrocytes to induce barrier properties in non-CNS blood vessels in transplantation studies,as well as induce barrier properties in cultured ECs in in vitro coculture paradigms.

One issue with these studies is that the astrocytes are often cultured from neonatal rodent brains and go through many rounds of cell division, suggesting that these studies are analyzing progenitor cells as opposed to mature astrocytes. Recent data analyzing the BBB in dissected rodent embryos suggest that the BBB is formed before astrocyte generation and ensheathment of the vasculature, and, thus, these cells do not play a role in the initial induction of the BBB. The identification of astrocyte-secreted factors that do regulate BBB function suggests that mature astrocytes modulate and maintain the barrier once it is formed.

4.5 Immune Cells

CNS blood vessels interact with different immune cell populations both within the blood as well as within the CNS. The two main cell populations within the CNS are perivascular macrophages and microglial cells. Perivascular macrophages are monocyte lineage cells that sit on the ablumenal side of the vascular tube commonly found in the Virchow–Robin space, a small fluid filled canal that lines the ablumenal surface of the veins and arteries that enter/leave the CNS.These cells are derived from blood-borne progenitors, and chimera experiments suggest that they are able to cross the BBB and can be 80% replaced within 3 mo.These cells provide a first line of innate immunity by phagocytosing cellular debris. Microglial cells are resident CNS parenchymal immune cells that are derived from progenitors in the yolk sac and enter the brain during embryonic development.These cells are involved in regulating neuronal development, innate immune response, and wound healing, and can act as antigen-presenting cells in adaptive immunity.In addition, different blood-borne immune cell populations, including neutrophils, T cells, and macrophages, can interact with CNS vessels when activated and are thought to regulate BBB properties in response to infection, injury, and disease by releasing reactive oxygen species that can increase vascular permeability. Identifying the mechanisms by which both the immune cells and the BBB become “activated” to interact may be important in deciphering the mechanisms by which the BBB is disrupted during different neurological diseases.

V. MOLECULES OF THE BLOOD BRAIN BARRIER

The discovery of molecules expressed by CNS ECs has led to the identification of important structural and transport components of the BBB (Fig.3).Recently, the use of large-scale genomic and proteomic experimental approaches has provided greater detail and understanding of the molecular biology of the BBB. Use of acutely purified microvascular fragments, acutely purified ECs, and cultured ECs combined with microarrary technology, RNA sequencing, and mass spectroscopy proteomic analysis have enabled large-scale gene expression comparisons of CNS ECs with neural cells as well as ECs from other tissues. In particular, comparison of the molecular differences between CNS ECs and ECs from nonneural tissues has provided an understanding of the unique molecular composition of the BBB.

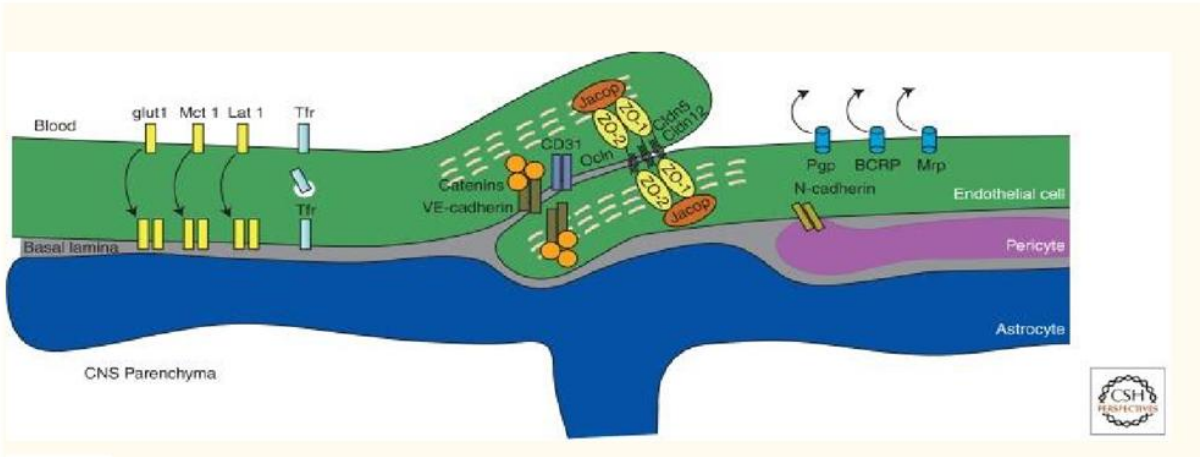

Figure 3

Schematic representation of molecules of the BBB. CNS, central nervous system; VEcad, VE cadherin.

5.1 Tight Junctions

CNS ECs are held together by TJs, which create a high-resistance paracellular barrier to molecules and ions, polarizing the luminal and ablumenal compartments. Most of what is known about TJs is from work on ECs, which have identified that these cellular adhesions are formed on the apical part of the lateral membrane by homotypic and heterotypic interactions of transmembrane molecules that are linked to the cytoskeleton through interactions with cytoplasmic adaptors. The strength of the junctions varies greatly depending on the tissue in which they are found, and work in cell culture suggests that they have a size-selective permeability to uncharged

molecules of up to 4 nm, and then low permeability to larger molecules.This suggests that the TJs form a 4-nm pore and that larger moleculeswould pass through discontinuities in the junctions.

The transmembrane molecules include claudins, occludins, and JAMs.Claudins are a class of more than 25 different family members that are tetraspanins characterized by a W-GLW-C-C domain in the first extracellular loop.Evidence in vitro suggests that claudins are essential for the paracellular barrier formation. Expression of claudins is sufficient to form TJ strands in fibroblasts, and disruption of claudins decreases the paracellular barrier properties of canine kidney cells.Work with chimeric claudins has shown that amino acid residues in the first extracellular loop define the size and charge selectivity of the pore within the cellular junction, and, thus, the composition of the claudins within a given cell can determine the permeability of the paracellular barrier.Different claudin family members are expressed by different epithelial barriers in different tissues, and mouse knockouts have shown that specific family members are essential for specific,many of which have been associated with human disease.Claudin-5 has been shown to be highly expressed by CNS ECs, and mice that lack claudin-5 have a size-selective leak of the BBB. In addition, other claudins, including cldn12 and cldn3, have been identified at the BBB.Occludin is a tetraspanin expressed by epithelial cells and CNS ECs, an in vitro culture experiment disrupting occludin homotypic interactions suggest that it is important for the resistance of the barrier. Occludin is highly enriched in CNS ECs compared with ECs in nonneural tissues, indicating that it may be an important component of the barrier. Occludin-deficient mice, however, are shown to have a normal high-resistance epithelial barrier and a functioning BBB. These mice do have calcification of the CNS suggesting that perhaps occludin specifically regulates calcium flux across the BBB.JAMs are immunoglobulin superfamily members that form homotypic interactions at tight junctions in epithelial cells and ECs. JAMs have been shown to regulate leukocyte extravasation as well as paracellular permeability.In particular, JAM4 has been identified at the BBB in mice.Recently, it has been shown that unique molecular components are required to seal the paracellular barrier at the contact points of three cells. These tricellular junctional complexes are made up of lipolysis-stimulated receptor (LSR), which is required to localize marveld2 to tricellular adhesions.It remains unclear what the nature of the size/charge-selective pore is formed by the specific composition tight junction proteins expressed by CNS ECs, and whether the permeability of this pore is static or whether it is dynamically altered in response to neuronal activity.

The transmembrane adhesion complexes are linked to the cytoskeleton through a series of cytoplasmic adaptors including ZO-1, ZO-2, Cingulin, Jacop, MAGIs, and MPPs.In addition, the TJs interact with basal adherens junctions (AJs), which connect all ECs and are made up of vascular endothelial (VE)- cadherin and platelet EC adhesion molecules (PECAM)1, and are linked to the cytoskeleton by catenins. Interestingly, many of the TJ proteins identified, including cldn5, cldn12, ZO-1, and ZO2, appear to be expressed by ECs in all tissues. Thus, a major question is why only CNS ECs form this tight barrier and not ECs in other tissues. Transcriptional analysis comparing CNS ECs with peripheral ECs suggests that several cytoplasmic adaptors, including jacop and MPP7, as well as tricellular TJ molecules, LSR and marveld2,are enriched at the BBB suggesting that these molecules may be criticalfor this barrier formation.

5.2 Transporters

CNS ECs are highly polarized cells that have distinct lumenal and ablumenal compartments. The low permeability of the paracellular junctions allows the transport properties of the cells to control the movement of ions and molecules between the blood and the brain. There are two main types of transporters expressed by CNS ECs: efflux transporters and nutrient transporters. Current work to elucidate the full array of transporters and their substrates is highly sought after both to understand the external requirements for brain metabolism and function, but also to identify targets to aid in drug delivery across the BBB.

Efflux transporters, including Mdr1, BCRP, and MRPs, use the hydrolysis of ATP to transport their substrates up their concentration gradient.Many of these transporters are localized to the lumenal surface and transport a wide array of substrates into the blood compartment. This wide substrate diversity allows these transporters to provide a barrier to many small lipophilic molecules, which would otherwise passively diffuse through the EC membrane. Mdr1, also called P-glycoprotein, has been widely studied in this context, and knockout mice show an increase in a wide variety of small lipophilic drugs entering the brain, as well as endogenous molecules.Up-regulation of Mdr1 has also been associated with drug-resistant epilepsy and tumors.An important avenue of research uses structural modeling to predict substrates of these efflux transporters to develop therapeutics that can avoid efflux and, thus, gain entry to the CNS. In addition, developing inhibitors of these efflux transporters is an ongoing research avenue to aid in delivery of small molecule compounds to the CNS. Interestingly, not much is known about the endogenous molecules that are effluxed by these transporters, how this is important to regulate brain function, and whether inhibitors would alter the tissue distribution of important endogenous molecules.

Nutrient transporters facilitate the movement of specific nutrients down their concentration gradient. CNS ECs express a wide variety of these transporters to deliver very specific nutrients across the physical

barrier of the CNS ECs into the CNS parenchyma. Many of these belong to the solute carrier class of facilitated transporters, including slc2a1 (glucose), slc16a1 (lactate, pyruvate), slc7a1 (cationic amino acids), and slc7a5 (neutral amino acids, l-DOPA).Slc2a1, also called glut1, has been largely studied for its role in providing the CNS with glucose. Expression of this transporter is highly enriched in CNS ECs compared with ECs in nonneural tissues, and it facilitates the transport of glucose down its concentration gradient from the blood into the brain.In humans, Glut1 deficiency leads to an epileptic syndrome that is treated by being fed a high-ketone diet.In addition, CNS ECs express a variety of different receptor-mediated transport systems, including the transferrin receptor (transferrin/iron), Ager (amyloid), and low-density receptor-related lipoprotein (LRP)1/LRP8. Many of these transport systems are being targeted as Trojan horses to aid in drug delivery to the CNS. Although most of these transporters provide nutrients from the blood to the brain,several are also important for removing waste products from the brain.A complete characterization of BBB transporters, their substrates, and their direction of transport is critical to determine the external nutrient requirements of the CNS and how the BBB mediates the interaction between the blood and the CNS. Recently, systemic proteins have been implicated in regulating neurogenesis differently in youth and during aging however, it remains unclear whether this is because of specific transport, localized permeability of the BBB, or nonspecific passive movement of small amounts of systemic factors.

5.3 Transcytosis

In CNS ECs, the rate of transcytosis is dramatically lower than in ECs in nonneural tissues but is up-regulated as a major component of BBB dysfunction during injury and disease. Transcytosis through ECs is mediated through caveolin-based vesicle trafficking. Caveolin-1 is expressed by all ECs and is up-regulated at the BBB following traumatic brain injury. Plasmalemma vesicle-associated protein (PLVAP) expression is enriched in peripheral ECs compared with CNS ECs, and has been implicated in vesicle trafficking, formation of fenestra, and leukocyte extravasation in these "leaky" vascular beds. This molecule is also up-regulated in CNS ECs in a variety of diseases in which there is BBB leakage.Therefore, lack of PLVAP in healthy CNS ECs appears to be important for limiting permeability.

5.4 Leukocyte Adhesion Molecules (LAMs)

In the healthy CNS, there is an extremely low level of immune surveillance, with an almost complete lack of neutrophils and lymphocytes within the parenchyma. Entry of a leukocyte from the blood into a tissue is a multiple-step process that includes rolling adhesion, firm adhesion, and extravasation. This requires a series of different leukocyte adhesion molecules, including selectins (E-selectin, P-selectin) for rolling adhesion and immunoglobulin family members for firm adhesion. The expression of these adhesion molecules is much lower in CNS ECs than in peripheral ECs but is elevated during neuroinflammatory diseases, such as stroke and MS.Interestingly, different subsets of inflammatory cells are observed infiltrating the CNS in different diseases. For instance, in MS, there is infiltration of T cells, B cells, neutrophils, and macrophages at sites of active lesions, whereas in stroke, there are neutrophil and macrophage infiltrates but lymphocytes are largely excluded. An important question is whether each cell has a different mechanism for crossing the BBB, and whether the discrimination is done at the level of the activated BBB or the activated immune cell.

5.5 Other Components of the BBB

Large-scale genomic and proteomic approaches have identified signaling cascades that are turned on in CNS ECs. In particular, Wnt/β-catenin signaling through Lef1, as well as Sonic hedgehog signaling through Gli have been shown to be important for regulating the formation and function of the BBB. In addition, vascular metabolism has been implicated in regulating barrier properties of CNS vasculature by metabolizing potential toxins or altering the properties of molecules (Fig.2). Specific enzymes, including carbonic anhydrase IV and γ-glutamyl transpeptidase, have been identified as enriched in CNS vessels compared with vessels from non-neural tissues.

Large-scale genomic and proteomic approaches have provided invaluable resources in understanding the gene expression of the BBB, but work still needs to be done to identify which of these BBB- enriched genes are important for each aspect of the BBB, whether there is heterogeneity of these genes at different segments of the vascular tree and in different brain regions, and whether the expression and function of each protein is dynamically regulated by neuronal function, stress, or diet. In addition, work expanding beyond genomics is aimed at identifying the proteomics, miRNAs, noncoding RNAs, lipids, metabolomics, epigenetics, and other regulatory steps that are important for BBB formation andfunction.

VI. REGULATION OF THE BBB FORMATION AND HOMEOSTASIS

Although key properties of the BBB are manifested within the ECs, important transplantation studies have shown that they are regulated by interactions with the microenvironment of the CNS. The BBB is not one

physiology, but a series of physiological properties that either need to be induced (TJs, transporters, metabolic enzymes) or inhibited (transcytosis, LAMs) in CNS ECs. Recent work has dissected the cellular and molecular mechanisms that regulate this process, and have identified that it is a complex process of induction and maintenance signaling interactions among CNS ECs and PCs, astrocytes, and immune cells.

6.1 Regulation of Barrier Properties during Angiogenesis

Recent work in genetic mouse models has shown that there is a unique angiogenic program driving vessel formation in the CNS regulated by Wnt/β-catenin that also induces specific barrier properties in CNS ECs.Comparative microarray analysis has identified that effectors of Wnt/β-catenin signaling, including Lef1, Apcdd1, and tnfrsf19, are enriched CNS ECs compared to ECs in peripheral organs.Transgenic reporter mice have confirmed that Wnt/β-catenin signaling is activated in CNS ECs during embryonic angiogenesis.Different Wnt ligands are secreted by neural stem cells and neural progenitors in spatially distinct regions, notably Wnt7a and Wnt7b in ventral regions and Wnt1, Wnt3, Wnt 3a, and Wnt4 in dorsal regions.Disruption of Wnt signaling in all ECs by conditional depletion of β-catenin leads to widespread CNS angiogenic defects with overtly normal blood vessel formation in peripheral tissues. These defects include a thickening of the vascular plexus, which contains endothelial progenitors, a loss of capillary beds, and the formation of hemorrhagic vascular malformations, which together suggest that Wnt is a migration signal driving vessels into the CNS.These phenotypes were also observed following deletion of neural Wnts (Wnt7a/7b), demonstrating that the CNS angiogenic program requires Wnt as well as β-catenin. This CNS-specific angiogenic program was also shown to induce the expression of nutrient transporters, such as glut1, as well as the specific tight junction molecules like claudin-3 .Taken together, these data suggest that specific properties of the BBB are induced as vessels invade the CNS by a unique angiogenic program. Different Wnt ligands and Fzd receptors are expressed in spatially distinct regions and appear to be important for the regulation of CNS angiogenesis and BBB formation in those regions. One interesting receptor/ligand pair is Norrin/Fzd4, which is required for the formation of the retinal vasculature. Norrin is a transforming growth factor (TGF)-β family member with no homology with Wnt ligands, which is able to activate Fzd4 and induce canonical Wnt signaling. Loss of Norrin or Fzd4 produces major retinal vascular defects including a reduction in endothelial proliferation, vascular malformations, crossing of arteries and veins, a loss of venous fate, and leakiness of the blood–retinal barrier.Fzd4 mutants also have regional-specific BBB defects in the cerebellum, spinal cord, olfactory bulb but not the cortex, striatum, or hypothalamus. The more widespread phenotype of Fzd4 mutants suggests that it may also be activated by other ligands. Use of genetic mosaics has shown that Fzd4 is required cell-autonomously for sealing the BBB, and the Fzd4-deficient ECs have a loss of claudin-5 and an increase in PLVAP.Interestingly, deletion of Fz4 in adults leads up-regulation of PLVAP, loss of claudin-5, and leakage of the BBB, whereas reintroduction of Norrin to Norrin-deficient retinas leads to sealing of BBB properties.These data suggest that canonical Wnt signaling is not only required for BBB induction, but that also for maintenance of the BBB phenotype in adults, when the ligands are glial derived.

6.2 Regulation of the BBB by Pericytes

Analysis of mouse mutants in PDGFBB-PDGFR-β signaling has identified an important role for PCs in regulating BBB formation and function.These mutant mouse models include Pdgfb null and Pdgfrb null mice that completely lack CNS PCs and die at birth, as well as extracellular matrix (ECM)- retention motif mutations to Pdgfb or hypomorphic alleles of Pdgfrb in which mice have fewer PCs than their wild-type littermates. Analysis of the BBB in Pdgfrb null mice during embryogenesis revealed a leaky BBB, demonstrating that PCs are required to regulate the formation of the BBB. In particular, lack of PCs leads to an increase in the rate of transcytosis and an increase in the expression of LAMs resulting in CNS-immune infiltration.Further use of mice with Pdgfrb hypomorphic alleles, which have varying numbers of CNS PCs, showed that the total number is important for the relative permeability of the vessels.Additionally, work done in adult mice with ECM-retention motif mutations to Pdgfb that contain fewer PCs has identified that PCs are required during adulthood to regulate BBB homeostasis, and particularly do so by inhibiting transcytosis .Microarray analysis comparing the transcriptional profile of CNS ECs with pdgfrb mutant mice and wild-type mice suggest minimal changes in the expression of genes involved in BBB-specific properties, such as tight junctions, nutrient transport, or efflux transport, but an increase in the expression of genes involved in peripheral EC-specific "leaky" properties, including transcytosis (PLVAP) and leukocyte adhesion.Taken together, these data suggest that PCs are not involved in the induction of BBB-specific properties (TJs, transporters), but play an important role in the inhibition of properties normally associated with leakyperipheral vessels (transcytosis, LAMs).

6.3 Regulation of the BBB by Astrocytes

The persistence of a functional BBB throughout adulthood is maintained and regulated by numerous factors unique to the microniche of the neurovascular unit (NVU) (Abbott et al. 2006). Astrocyte– BBB–EC interactions are known to regulate EC morphology, angiogenesis, and to influence the phenotype of the barrier

under physiological and pathological conditions.

Astrocytes are known to produce factors that modulate endothelial functioning during development and adulthood. One of these pathways is the Hedgehog (Hh) signaling cascade known to be involved in embryonic morphogenesis, neuronal guidance, and angiogenesis. Astrocytes secrete Sonic Hh (SHh),and BBB ECs express the Hh receptor Patched-1, the signal transducer Smoothened (Smo), as well as transcription factors of the Gli family. Interestingly, transendothelial electrical resistance (TEER) and permeability experiments showed that activation of the Hh pathway induced expression of junctional proteins and promoted a BBB phenotype. In addition, mice genetically engineered to lose the signal transducer Smo on ECs had a significant increase in BBB permeability that correlated with a decrease in junctional protein expression and disturbed BMs (Alvarez et al. 2011a, 2013), supportingthe concept that the Hh pathway has a significant influence on BBB function.

Astrocytes also secrete angiogenic factors that promote vascular growth, such as vascular endothelial growth factor (VEGF). During development, VEGF is required for the formation, remodeling, and survival of embryonic blood vessels. During early embryogenesis, radial glia cells seem to be the source of VEGF needed for vascular development, although ECs have been described to promote cell- autonomous activation of the VEGF signaling.Although VEGF is a factor mostly known to promote angiogenesis during development, in adulthood, VEGF decreases the stability of the BBB during inflammatory conditions.

Perivascular cells, including astrocytes, secrete angiopoietins (Ang1), which participate in the complex process of BBB differentiation by promoting angiogenesis and inducing a time-dependent decrease in endothelial permeability. This occurs through the up-regulation of junctional protein expression.In contrast, Ang-2 is known to participate in the early phases of BBB breakdown during injury and disease.Interestingly, when factors known to compromise BBB function, such as VEGF, are coexpressed with Ang1, the barrier integrity is enhanced and neuroprotective properties are induced (Shen et al. 2011).

Astrocytes also produce the angiotensin-converting enzyme-1 (ACE-1), which converts angiotensin I into angiotensin II and acts on type 1 angiotensin receptors (AT1) expressed by BBB ECs. Angiotensin II induces tightening of vessels, and, in the CNS, activation of AT1 restricts BBB permeability and stabilizes junctional protein function by promoting their recruitment into lipid rafts. Angiotensinogen (AGT)-deficient mice have an aberrant expression of occludin at the BBB, suggesting that astrocyte- secreted angiotensin II promotes TJ formation.

TGF-β is a pleiomorphic cytokine involved in cell growth, differentiation, morphogenesis, apoptosis, and immunomodulation. In the CNS, TGF-β is neuroprotective, and in vitro studies have shown its capacity to induce Mdr1 activity and to reduce BBB permeability (Dohgu et al. 2004). TGF-β is secreted by astrocytes and CNS-ECs, and TGF-β known to down-regulate the extent of leukocyte transmigration across the endothelium. However, the overwhelming pleomorphic roles of TGF-β do not currently allow a conclusion on the exact role of astrocyte-derived TGF-β in BBB physiology.

Retinoic acid (RA) can be secreted by radial glial cells, and recent findings suggest that RA is also secreted by astrocytes, and its receptor, RA-receptor β (RAR-β), is expressed in the developing vasculature. RAR-β activation increases TEER, which correlated with enhanced expression of VE- cadherin, P-gp, and ZO-1. In vivo, pharmacologic modulation of RAR-β resulted in a perturbed BBB.Interestingly, RA is known to regulate the Hh, Wnt, and FGF pathways,which implies that RA secretion by radial glial cells could be a master upstream regulator of BBB development.

6.4 Convergence of Signaling Events at the BBB

It is clear that the BBB is regulated by a complex set of cellular signaling mechanisms that regulate both the induction of barrier properties during initial angiogenesis into the CNS, as well as the maintenance of barrier properties in adults. Neural stem cells appear to be the key cell type involved in the early differentiation of the ECs into BBB ECs, and then PCs and astrocytes provide further cues modulating the different barrier properties of these CNS ECs. The number of distinct factors that are known to impact on BBB permeability highlights the diversity of the CNS inputs needed to generate this physiological barrier. This also emphasizes the redundancy of molecular signals affecting BBB formation and stability, and the need for future work to identify how each of these signals are coordinated to regulate different aspects of the BBB. These signals can, however, be integrated into a general concept.Key signaling pathways and transcription factors have either barrier-promoting properties (Wnt, Hh, Sox-18, nrf-2, ERG, Nkx2-1, and SP3/YY1) or barrier-disrupting effects (NF- κB, Snail, FoxO1, PKC, and eNOS). Within the signaling pathways promoting BBB functioning, Wnt and Hh seem to be dominant and to cooperate in driving a BBB phenotype. Wnt ligand binding to Frizzled/LRP5/6 activates β-catenin, which leads to the expression and targeting of the junctional proteins claudin-3 and p120 to the cell membrane. β-Catenin also down-regulates the activity of Snail, which has a negative effect on the stability of p120/VE-cadherin complexes and on the expression of TJ molecules occludin and claudin-5. Loss of the Wnt coreceptor Lrp5 causes down-regulation of claudin-5 expression.The Hh signaling pathway appears to drive the transcription and expression of junctional proteins, but also dampens inflammatory responses on CNS-ECs. Activation of Gli-1 by the Hh ligands or wnt signaling are reported to activate Sox-18,which control

claudin-5 expression.Wnt and Hh activation also induce the expression of NR2F2, a transcription factor that promotes Ang-1 expression, inducing junctional protein expression through tie-2. NR2F2 also down-regulates expression of Ang-2, a factor known to decrease junctional protein expression. In a similar way, activation of the nrf-2 pathway by oxidative stress activates antioxidant response elements (ARE), which are known to stabilize ZO-1, occludin, and claudin-5 expression.In addition, nrf-2 protects ECs during injury by supressing the expression of inflammatory genes.In this sense, signaling pathways and transcription factors supporting barrier function also tend to promote anti-inflammatory responses.

One of the major issues when analyzing previous work is that many different measures have been used to quantify BBB function when analyzing the effect of genetic or environmental perturbations on the barrier, making it difficult to compare and contrast different studies. Furthermore, in many cases, only a small number of measures are used to examine BBB function, whether a single molecular tracer or analysis of a small set of molecular markers. The BBB is not a single entity, but a series of different properties possessed by the CNS ECs and regulated by interactions with different neural, vascular, and immune cells; thus, a more exhaustive approach to understanding how different pathways regulate each aspect of the BBB is required to fully understand this barrier. Thus, future work needs to identify whether each of these signaling pathways regulate all aspects of the BBB, or whether different properties of the BBB are induced and regulated by different pathways, and, if so, how do each of these pathways coordinate to regulate the BBB, allowing proper neuronal function. New genetic tools allow for manipulation of genes and pathways both in development and in adulthood and, thus, will be able to determine whether the pathways are required for induction during development, maintenance during adulthood, and/or disruption during disease. Furthermore, new intravital imaging techniques in live awake-behaving animals will enable the understanding of how plastic the BBB is and whether different properties of the BBB can be dynamically regulated in response to neuronal activity, diet, infection, or other environmental stimuli.

VII. DYSFUNCTION OF THE BBB IN CNS DISORDERS

Disruption of the BBB is observed in many different neurological disorders including MS, stroke, Alzheimer's disease (AD), epilepsy, and traumatic brain injuries. Functional imaging of human patients and analysis of postmortem brain samples has identified the pathological breakdown of the barrier in different neurological diseases. In addition, work with animal models of disease and with cell culture BBB models has enabled the identification of some of the molecular mechanisms that cause changes to the BBB. This dysfunction can include alterations in many different properties of the BBB including TJs, transporters, transcytosis, and LAM expression. This breakdown can lead to edema, disruption of ionic homeostasis, altered signaling, and immune infiltration that can lead to neuronal dysregulation and, ultimately, degeneration. Although BBB dysfunction is often secondary to the primary insult in these diseases, in some cases, it has been a suggested cause, including MS,epilepsy, and AD (Fig.4).

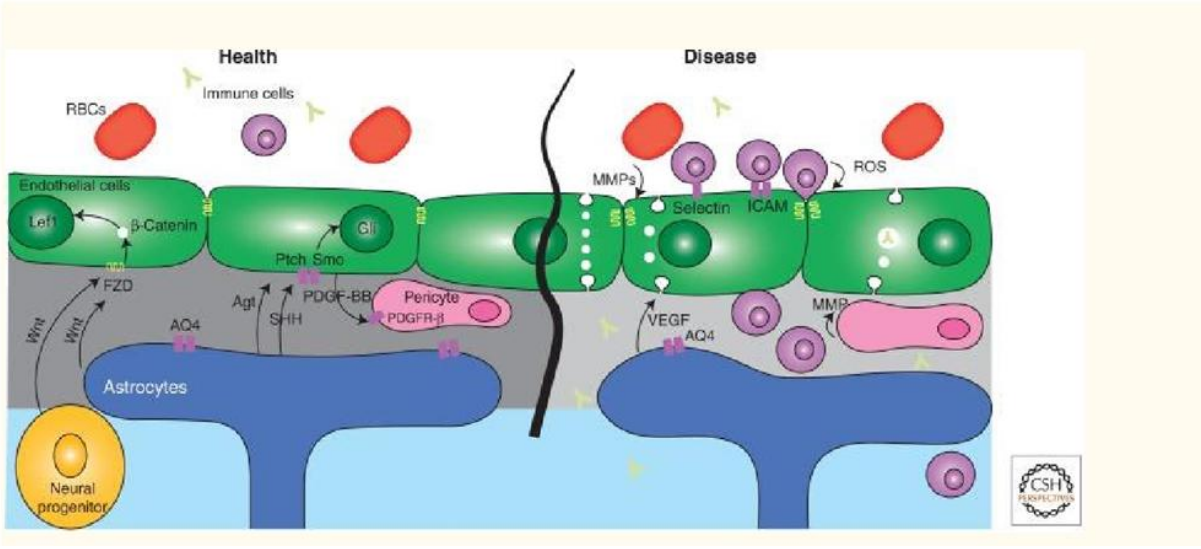

Figure 4

Schematic representation of signaling regulating the blood–brain barrier in health and disease. ICAM, intercellular adhesion molecule; MMP, matrix metalloproteinase; ROS, reactive oxygen species.

7.1 MS and Related Disorders

In most CNS pathologies, the BBB is affected as a result of the inflammation, injury, or degenerative processes specific to the pathology. However, in only a few diseases, the BBB is specifically targeted by the pathogenic process or by the disease determinants. Neuromyelitis optica (NMO) and MS are among these diseases. Astrocytes are generally not regarded as a primary target of the immune system in MS, although BBB disruption and alterations in astrocyte physiology are hallmarks of MS pathogenesis. The etiology of MS remains elusive, but it is clear that multiple factors are involved in disease development, including environmental and genetic factors. Nevertheless, MS is a T-cell- mediated disease in which CD4 T-helper (Th) cells of the Th17 and Th1 phenotype play a fundamental role in its pathogenesis. B cells are also essential in MS immunopathogenesis, as antibodies produced within the CNS are a fundamental feature of the disease (i.e., oligoclonal bands) and as B-cell-directed therapies provide strong protection against lesion formation. It is clear that during immune cell infiltration and lesion formation, BBB function becomes compromised,which is characterized by vascular leakage associated with alterations of junctional proteins. Analysis of MS tissue shows that abnormalities in the expression of junctional proteins coincide with perivascular astrogliosis, and such changes are detected in very early stages of lesion formation.This has been, in part, explored by Luo et al. (2008) when inducing active experimental autoimmune encephalomyelitis (EAE) in mice expressing luciferase under the control of GFAP. Despite showing clinical signs only at day 11, increases in bioluminescence associated with GFAP expression could be detected in the brain of these animals as early as 3 d postinduction suggesting that astrocytes are activated in the very early stages of EAE and in the absence of clinical signs of the disease.

Besides its primary neuroprotective function, the BBB has also been shown to actively promote neuroinflammation by orchestrating immune responses during CNS-targeted autoimmune aggression. BBB ECs are an important source of proinflammatory chemokines CCL2,CCL5, and CXCL10, which are required for lymphocyte and monocyte recruitment to the CNS.Immune cell infiltration into the CNS correlates with production of proinflammatory mediators, such as interleukin (IL)-17, IL-22, granulocyte macrophage colony–stimulating factor (GM-CSF), interferon (IFN)-γ, and tumor necrosis factor (TNF).These cytokines have been implicated in the modulation of EC function by up-regulating the expression of proinflammatory mediators and by affecting the expression of junctional proteins and, thus, compromising BBB permeability. Last, BBB ECs express intercellular adhesion molecule (ICAM)-1, ICAM-2, vascular CAM (VCAM)-1, activated leukocyte CAM (ALCAM), melanoma CAM (MCAM), and Ninjurin-1, which mediate, at least in part, the adhesion process and transmigration of leukocytes and leukocyte subtypes to the CNS.Thus, although the BBB protects against CNS-directed inflammation by restricting immune cell access to the brain, it can also regulate the local inflammatory response by expressing proinflammatory molecules that promote the recruitment of peripheral immune cells into the CNS.

Following migration across ECs, leukocytes cross the endothelial BM and, subsequently, the parenchymal BM to get access into the CNS. The composition of the endothelial BM can regulate the extent of perivascular infiltration as large amounts of leukocytes are detected in vessels expressing laminin 411 and low levels of 511, whereas in the absence of 411, laminin 511 is ubiquitously expressed and associates with low-T-cell infiltration and milder disease. In EAE and MS, immune cell infiltrates are in great part contained to the perivascular space, and the process of leukocyte migration across the parenchymal BM and astrocyte endfeet appears to be more tightly controlled than the diapedesis across ECs.In EAE, CD4+ T-cell infiltration across the parenchymal BM is not laminin dependent, but rather requires focal activation of and matrix metalloproteinases (MMP)-2 and MMP-

9 to selectively cleave dystroglycan, affecting the BM stability and integrity.Interestingly, parenchymal BM components and other ECM-binding receptors on the astrocyte endfeet remain unaffected, indicating the existence of specific and specialized protective mechanisms under the control of astrocytes and possibly other cells within the NVU.Thus, further understanding is needed in terms of astrocyte involvement in supporting or inhibiting the activation and migration of immune cells as well as the repair of the affected BBB/NVU during MS/EAE and other CNS disorders.

Reactive astrocytes can also be the source of factors that will negatively affect barrier function at the NVU. In MS and EAE, VEGF-A is expressed by reactive astrocytes, and in vitro/in vivo studies showits capacity to induce BBB breakdown by disrupting claudin-5 and occludin expression and promote immune cell infiltration to the CNS. Additional studies propose that IL-1 production by microglia induces VEGF-A up-regulation. VEGF-A is released from the astrocytes and binding to its receptor VEGFR2 on BBB-ECs activates eNOS-dependant down-regulation of the junctional proteins claudin- 5 and occludin that leads to BBB breakdown.

Although reactive astrocytes can produce BBB-promoting (i.e., Hh) or BBB-disrupting (i.e., VEGF) factors, they can also lose or down-regulate factors that have the capacity to promote barrier function. In this regard, astrocytes produce AGT (which is cleaved into angiotensin II), and analysis of MS tissues showed that expression of AGT in astrocytes and occludin in ECs is decreased in MS lesions when compared to normal

appearing white matter. This pattern correlates with the down-regulated expression of AGT detected in astrocytes stimulated in vitro with IFN-γ and TNF-α. Interestingly, nonimmunized (non-EAE) AGT-deficient mice have compromised BBB function, which correlates with decreased and disrupted expression of occludin. Therefore, local inflammatory mediators present in perivascular cuffs can also negatively impact on the capacity of reactive astrocytes to promote BBBfunction by down-regulating their production of BBB-promoting factors.
NMO is also an immune-mediated disease of the CNS affecting predominantly the spinal cord and the optic nerves. In NMO, the production of anti-AQP4 IgG antibodies affects the function of the astrocyte water channel AQP4 directly affecting BBB function. Binding of anti-AQP4 antibodies to their target results in the activation of complement-dependent cytotoxic cell damage that leads to the loss of AQP4, GFAP, and the excitatory amino-acid transporter 2 (EAAT2). In addition, the BBB damage is associated with focal areas of perivascular immune cell infiltration and demyelination, particularly granulocytes, and eosinophils that degranulate in the perivascular space causing local damage that includes astrocyte injury. Although oligodendrocytes are affected as a result of the pathophysiological changes, the exact mechanism(s) leading to oligodendrocyte and neuronal damage remains to be determined.

7.2 Modulation of the BBB following Hypoxia/Ischemia and in Stroke

In vivo and in vitro stroke models have shown that cerebral vascular permeability increases in a time- and hypoxia-dependent manner. This leads to a subsequent increase in cerebral edema; however, the processes involved in the hypoxia-induced BBB permeability are not completely understood. Work in animal models of stroke has identified that there is a biphasic leakage of the BBB, with an early opening within hours following hypoxia/ischemia, followed by a refractory phase and then a second opening the next day.In addition, analysis in transgenic models has identified that there are stepwise alterations in the BBB, with an increase in transcytosis observed first followed by alterations in the TJs.There are also important changes in ion channel and efflux transporter expression and activity.

Focal cerebral ischemia damages elements of the BBB and induces inflammatory processes that alter the relationships of ECs, ECM, and astroglial cells. This results in profound changes in the microvascular permeability barrier. Focal increases in permeability to fibrinogen, IgG, and other large proteins are detected within a few hours following middle cerebral artery occlusion (MCAO). Conversely, and surprisingly, hypoxic conditions induce expression of ZO-1 in vitro and claudin-5 and occludin in vivo. The exact functional consequences of these up-regulations are not clear. Nevertheless, levels of EC-expressed integrins α1β1, α3β1, and α6β1 decrease rapidly after MCAO and MMPs are activated on ischemic insult, which induces basal lamina remodeling, and also chemokine activation. Finally, dystroglycan, expressed by astrocyte, disappears after MCAO, a phenomenon responsible for detachment of astrocyte endfeet and perivascular edema. These studies suggest that adhesive interactions between the endothelium and the ECM contribute to the acute vascular remodeling seen in stroke.

7.3 Molecular Alterations of the Tight Junctions

Clinically, strokes are known to cause an increase in vasogenic edema, which can be attributed to an increase in BBB permeability. Recent in vitro studies have begun to elucidate the molecular changes leading to increases in BBB permeability. In studies by Mark and Davis (2002) and Witt et al. (2008), an increase in actin protein levels and actin stress fibers was observed following hypoxic insult, whereas hypoxia alone had no effect on protein expression of the TJs, occludin, claudin-1, or ZO-1/2. Following hypoxia, reoxygenation increases expression of occludin, claudin-1, and ZO-1/2. Changes in the cellular localization of the TJ proteins occludin and ZO-1/2 following hypoxic insult were confirmed with dynamic confocal microscopy recordings. Interestingly, these changes were reversibleand returned to control levels on reoxygenation.

Changes in junctional structure formation or stability are now known to involve up-regulation in vascular endothelial growth factor (VEGF), and inhibition of VEGF attenuates the hypoxia-induced increase in BBB permeability.In addition, hypoxia increases nitric oxide (NO) release by ECs and inhibition of NO synthase reduces the effect of hypoxia on cell permeability. Although the exact mechanisms involved in the VEGF- and NO-mediated changes in EC permeability are still being investigated, some reports have shown that NO may directly modify the TJ proteins by nitrosylation or nitrosation.

7.4 MMPs and the BBB

MMPs are zinc-dependent proteases that have the ability to degrade fibronectin and laminins. As the basal lamina is composed of collagen, fibronectin, laminin, and heparin sulfate, and serves as an important scaffold for brain ECs, MMPs have been considered as obvious initiators of BBB disruption. Following ischemia/reperfusion, MMPs have been shown to be up-regulated in the brain,either through proinflammatory cytokine pathways (via NF-κB) or through activation of HIF-1α and furin, which

convert pro-MT-MMP into activated MT-MMP. More specifically, it has been shown that MMP-9, MMP-3, and MMP-2 levels were increased following ischemia/reperfusion, correlating with the increase in sucrose diffusion across the BBB. Additionally, inhibition of MMP with pharmacological agents or use of MMP knockout animals reduced BBB disruption.It remains unclear whether MMP- mediated BBB disruption occurs at the level of the basal lamina, or at the level of the TJ and AJ, as these junctional proteins were also shown to be substrates of MMPs.

7.5 Modulation of Channels and Transporters

Ion channels and transporters are key components of the BBB, which maintain cerebral physiological and metabolic homeostasis. As one of the major consequences of stroke is the formation of cerebral vasogenic and cytotoxic edema, understanding the effect of stroke on the function of channels and transporters at the BBB could identify important therapeutic targets.

During ischemic stroke, there is an important release of glutamate from neurons that bind to *N*-methyl-d-aspartate (NMDA) receptors. This excess NMDA receptor activation is largely responsible for cytotoxic edema of neurons.Studies have shown that BBB ECs also express both NMDA and metabotropic glutamate receptors.Circulating inflammatory mediators have also been shown to stimulate a release of glutamate, which disrupts the BBB via metabotropic receptors.Interestingly, in vitro studies showed that NMDA receptor activation reduces BBB integrity, whereas activation of metabotropic receptors increased BBB electrical resistance suggesting a tightening of the BBB.

The activity of exchangers and transporters, such as the Na+/H+ exchanger (NHE), Na+/K+ ATPase, and Na+/K+/Cl− cotransporter, contribute to maintaining ion balance at the BBB and in the brain in general. During stroke, osmotic and ion balance are altered, leading to activation of ion transporters and exchangers.

7.6 Regulation of the BBB in AD

Dysfunction of cerebral vascular ECs and leukocyte transmigration across the BBB probably participate in the development of AD, Parkinson's disease (PD), and other neurodegenerative diseases. As multidrug resistance function at the level of the BBB decreases with age, decreased clearance of neurotoxic compounds and increased oxidative stress in the brain increases the risk of neurodegenerative pathology.

Arterial spin labeling magnetic resonance imaging (MRI), functional blood-oxygen-level-dependent (BOLD)-MRI, fluorodeoxyglucose-positron emission tomography (FDG-PET), and single-photon emission computerized tomography (SPECT) studies in humans show that cerebral blood flow is significantly reduced first in mild cognitive impairment and then in AD. Amyloid β is transported from the blood to the brain by the receptor for advanced glycation endproducts (RAGE), which is expressed on BBB-ECs. Conversely, both soluble LRP and ApoE are cell-surface Aβ chaperones that associate with clearance receptors and promote extrusion of Aβ from the brain back into the blood through the BBB. In AD, these clearance pathways seem to be altered, which is hypothesized to lead to accumulation of soluble Aβ in the perivascular space and the formation of toxic oligomeric Aβ. Aβ deposition in the vascular smooth muscle cell layer and Aβ plaque formation around vessels of AD patients has been well documented, and participate in the pathology of cerebral amyloid angiopathy, an entity strongly linked to AD. Soluble amyloid β is also known to stimulate the transmigration of monocytes, to enhance tau pathology, to induce secretion of proinflammatory cytokines (TNF and IL- 6) and chemokines, to activate MT1-MMP, the activator of MMP-2, to stimulate production of MMP- 9, and to activate production of reactive oxygen species (ROS) when injected by microdialysis in vivo. Patients with AD have also been reported to have focal vascular defects in the CNS, such as vascular "regression," reduced capillary density, accumulation of collagen, perlecan in the basal lamina, reduced mitochondrial content, and loss of TJ and AJ proteins. These might well be caused by Aβ accumulation and BBB dysfunction, although it has not been proven.

VIII. CONCLUSIONS

The BBB is an important cellular barrier that tightly controls the microenvironment of the CNS to allow for proper neuronal function. This barrier is an extremely important factor to consider when determining treatments for different neurological diseases, both because disruption of the BBB can lead to severe pathology observed in many different diseases, but also because crossing the BBB is an essential consideration in the development of CNS-acting therapeutics. Recent work has identified many molecules required for BBB function as well as many of the cellular and molecular signaling events that regulate the formation of the BBB during development, its function in adulthood, and its response to injury and disease (Fig.3). Although much progress has been made, many questions still remain. Are all of the different properties of the BBB regulated by the same pathways or different pathways? How are different signaling pathways coordinated to regulate different aspects of the BBB? Which pathways induce properties of the BBB during development, and which are required throughout life for maintenance of the barrier? How dynamic is the BBB? Are different BBB properties, including

the transport and tight junctions, dynamically regulated in response to neural activity? How do alterations in the BBB affect neuronal activity, brain function, and behavior? Are there localized specialties of the BBB that regulate regional neuronal development or function? What leads to loss of BBB properties during neurological disease, a loss of maintenance signals or the presence of disruption signals? Understanding these questions will allow for the development of therapeutics to modulate the BBB both to restore its function during neurological disease and to develop methods to bypass the BBB for drug delivery (Fig.4).

REFERENCES

[1]. Abbott NJ. 2002. Astrocyte-endothelial interactions and blood-brain barrier permeability. J Anat 200:629–638 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
[2]. Adams S, Brown H, Turner G. 2002. Breaking down the blood-brain barrier: signaling a path to cerebral malaria? Trends Parasitol 18:360–366 [Google Scholar]
[3]. Aono S, Nakagawa S, Reynolds AB, Takeichi M. 1999. p120(ctn) acts as an inhibitory regulator of cadherin function in colon carcinoma cells. J Cell Biol 145:551–562 [Google Scholar]
[4]. Avgoustakis K. 2004. Pegylated poly(lactide) and poly(lactide-co-glycolide) nanoparticles: preparation, properties and possible applications in drug delivery. Curr Drug Deliv 1:321– 333 [Crossref], [PubMed], [Google Scholar]
[5]. Balda MS, Flores-Maldonado C, Cereijido M, Matter K. 2000. Multiple domains of occludin are involved in the regulation of paracellular permeability. J Cell Biochem 78:85–96 [Google Scholar]
[6]. Bartus RT, Snodgrass P, Marsh J, Agostino M, Perkins A, Emerich DF. 2000. Intravenous cereport (RMP-7) modifies topographic uptake profile of carboplatin within rat glioma and brain surrounding tumor, elevates platinum levels, and enhances survival. J Pharmacol Exp Ther 293:903–911 [Google Scholar]
[7]. Batycky RP, Hanes J, Langer R, Edwards DA. 1997. A theoretical model of erosion and macromolecular drug release from biodegrading microspheres. J Pharm Sci 86:1464– 1477 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
[8]. Bazile DV, Ropert C, Huve P, Verrecchia T, Marlard M, Frydman A, et al. 1992. Body distribution of fully biodegradable [14C]-poly(lactic acid) nanoparticles coated with albumin after parenteral administration to rats. Biomaterials 13:1093–1102 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
[9]. Bazzoni G. 2006. Endothelial tight junctions: permeable barriers of the vessel wall. Thromb Haemost 95:36–42 [Google Scholar]

Paduri Hrishitha. "Blood Brain Barrier." *IOSR Journal of Engineering (IOSRJEN)*, 12(03), 2022, pp. 16-29.