Trends in Neurosciences

Special Issue: Neuroimmunology

Review

The Molecular Constituents of the Blood–Brain Barrier

Brian Wai Chow and Chenghua Gu*

The blood–brain barrier (BBB) maintains the optimal microenvironment in the central nervous system (CNS) for proper brain function. The BBB comprises specialized CNS endothelial cells with fundamental molecular properties essential for the function and integrity of the BBB. The restrictive nature of the BBB hinders the delivery of therapeutics for many neurological disorders. In addition, recent evidence shows that BBB dysfunction can precede or hasten the progression of several neurological diseases. Despite the physiological significance of the BBB in health and disease, major discoveries of the molecular regulators of BBB formation and function have occurred only recently. This review highlights recent findings describing the molecular determinants and core cellular pathways that confer BBB properties on CNS endothelial cells.

Trends

The BBB comprises CNS endothelial cells that display specialized molecular properties essential for BBB function and integrity.

These molecular BBB properties are not intrinsic to CNS endothelial cells but have to be induced by the environment.

The formation, function, and maintenance of the BBB require functional interaction between CNS endothelial cells and NVUs.

Advances in gene profiling and cell-type purification methods have progressed the identification of the molecular mediators and core cellular pathways involved in BBB function and integrity.

A comprehensive understanding of the key molecules and cellular pathways involved in BBB function would offer novel strategies for CNS therapeutics.

History of the BBB

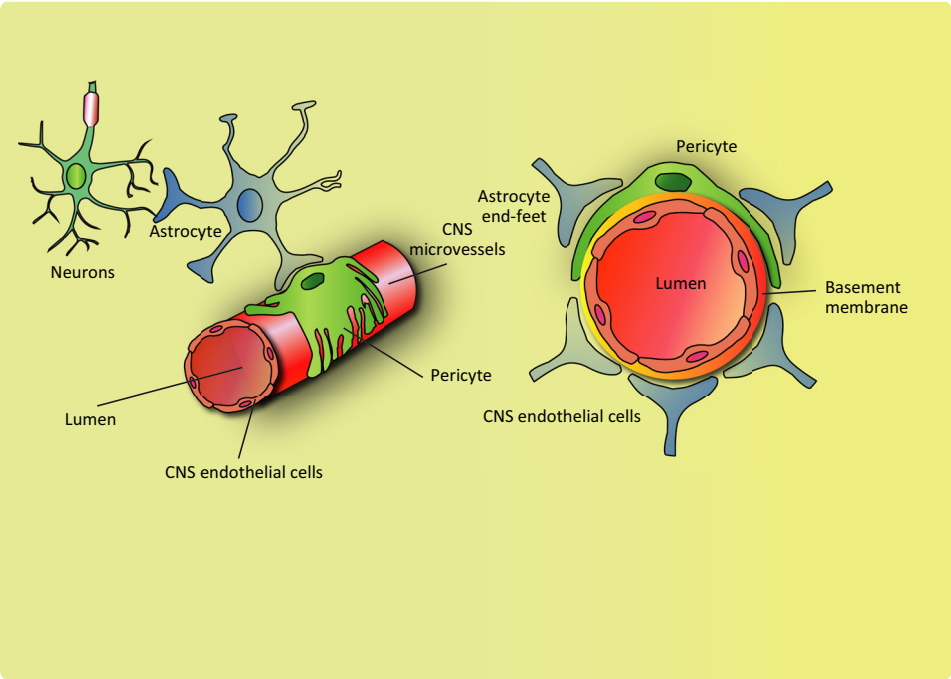
The **BBB** (see Glossary) partitions the brain from circulating blood and functions to: (i) shield the brain from potential blood-borne toxins; (ii) meet the metabolic demands of the brain; and (iii) regulate the homeostatic environment in the CNS for proper neuronal function [1]. The functional BBB comprises CNS **endothelial cells**, pericytes, astrocytes, and neurons that collectively form a functional '**neurovascular unit**' (NVU) (Figure 1) [2].

The BBB was first observed over a century ago. Pioneering physiologists studying the cerebrospinal fluid (CSF) noticed that water-soluble dyes injected in the peripheral circulation stained several tissues except the brain [3]. Ehrlich argued that this phenomenon occurred because the CNS had low affinity for the dye [4]. However, Goldmann questioned this argument, as injection of the same dyes in the subarachnoid space colored the brain but not peripheral tissues [5]. Continuing from these studies, Lina Stern and colleagues performed experiments in which they injected several vehicles into the brain parenchyma and blood. The results from these dye studies prompted Stern to introduce the term 'blood–brain barrier' and suggest its physiological function in maintaining brain homeostasis [6]. Over the years, the concept of the BBB fascinated physiologists but the anatomical site of the BBB was highly disputed; specific possibilities included the endothelium, astrocytic end-feet, and the basement membrane. A seminal study by Reese and Karnovsky using electron microscopy (EM) and injection of electron-dense horseradish peroxidase (HRP) resolved this dispute [7]. In this work, ultrastructural analysis by EM was used to delineate astrocytic end-feet and the luminal, abluminal, and basement membrane. Results revealed that the HRP was confined to the lumen of the CNS endothelium. Furthermore, EM revealed that the CNS endothelial cells are joined continuously by **tight junction** complexes and have limited intracellular vesicles [7]. Similar to Goldman's experiments, HRP injected into the brain parenchyma diffused past astrocytic end-feet and halted at the abluminal membrane of the endothelium, demonstrating that astrocytic end-feet do not significantly contribute to the physical barrier [8]. Thus, the site

Department of Neurobiology, Harvard Medical School, 220 Longwood Avenue, Boston, MN 02115, USA

*Correspondence: chenghua_gu@hms.harvard.edu (C. Gu).

 http://dx.doi.org/10.1016/j.tins.2015.08.003



Trends in Neurosciences

Figure 1. The Functional Blood–Brain Barrier (BBB) is Dependent on the Neurovascular Unit (NVU). The BBB is localized at central nervous system (CNS) microvessels, comprising a single layer of continuous, non-fenestrated endothelial cells. Surrounding the abluminal surface of the CNS endothelial cells are the basement membrane, pericytes, and astrocyte end-feet, collectively known as the NVU. BBB properties are not intrinsic to CNS endothelial cells but require continuous functional interactions with the NVU.

of the BBB is CNS capillaries comprising a single, non-fenestrated, continuous endothelial cell layer.

Molecular Properties of the BBB

CNS endothelial cells are highly polarized with distinct luminal (apical) and abluminal (basolateral) compartments [9]. The polarized nature of CNS endothelial cells is reflected in their four fundamental barrier properties that contribute to BBB function and integrity (Figure 2) [10]. First, circumferential tight junction complexes at the lateral, apical membrane between CNS endothelial cells establish a high-resistance paracellular barrier to small hydrophilic molecules and ions [8,11]. Tight junction complexes comprise: (i) tight junction proteins such as claudins and occludin; (ii) adhesion molecules such as VE-cadherin and E-cadherin; and (iii) junctional adhesion molecules [12,13]. These transmembrane proteins are further linked and stabilized to the cytoskeleton via multiple cytoplasmic adaptor proteins such as zonula occludens proteins [14]. Emerging studies have demonstrated that there is significant crosstalk among these tight junction complex proteins to regulate the restrictive barrier junction [15]. Second, in contrast to the peripheral endothelium, CNS endothelial cells display minimal vesicular trafficking, limiting the vesicle-mediated transcellular movement of cargo known as **transcytosis** [16]. Although CNS endothelial cells display limited transcytosis, it remains the preferred pathway for the selective transport of plasma macromolecules such as albumin and low-density lipoprotein [17]. Third, the establishment of the restrictive paracellular and transcellular barriers allows CNS endothelial cells to use highly polarized cellular transporters to dynamically regulate the influx of nutrients and efflux of metabolic waste and toxins between the blood and the brain parenchyma. The major class of known efflux transporters is the ATP-binding cassette (ABC) transporters – including Pgp, BCRP, and MRP – mostly localized at the luminal membrane [18–20]. These efflux

Glossary

Angiogenesis: the development of new vessels from proliferation of pre-existing endothelial cells.
Blood–brain barrier (BBB): a physiological barrier comprising a thin layer of continuous, non-fenestrated CNS endothelial cells that regulates the brain microenvironment for proper neuronal function.
Endothelial cells: mesoderm-derived cells that line the vasculature of the circulatory system.
Immune privilege: the introduction of antigens without eliciting an inflammatory adaptive immune response.
Neurovascular unit (NVU): the functional interactions among neurons, glia, pericytes, and endothelial cells.
Tight junction: a junctional complex between two cells that is essential for cell polarity, barrier functions, and cell adhesion.
Transcytosis: vesicular trafficking from the luminal to the abluminal plasma membrane and vice versa.