The Blood–Brain Barrier and the Gut–Brain Axis

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A fundamental characteristic of living organisms is their ability to separate the internal and external environments, a function achieved in large part through the different physiological barrier systems and their component junctional molecules. Barrier integrity is subject to multiple influences, but one that has received comparatively little attention is the role of the commensal microbiota. These microbes, which represent approximately 50% of the cells in the human body, are increasingly recognized as powerful physiological modulators in other systems, but their role in regulating barrier function is only beginning to be addressed.

Keywords: gut epithelium ; epidermis ; blood-brain barrier

1. Introduction

In contrast to the intestinal epithelium and the skin which bear their own microbiota and are thus exposed directly to microbial actions, the blood–brain barrier (BBB) represents a sterile physiological interface. Despite its physical separation from microbes however, there is increasing evidence that the BBB is a significant target for microbial actions, representing an important interface in microbe–brain communication, sometimes termed the gut microbiota–brain axis.

2. Structural Elements of the BBB

As the primary interface between the brain and the circulation, the BBB acts as a gatekeeper for blood-borne cells and molecules, protecting the delicate micro-environment of the brain tissue from their undue influence, as occurs in numerous metabolic and inflammatory diseases, including such major conditions as stroke, Alzheimer's disease and multiple sclerosis [1][2]. This vital function is ultimately dependent upon the complex structure of the BBB, formed as it is from several distinct but integrated elements. The primary face of the BBB, the cerebromicrovascular endothelium, is similar in many ways to other endothelia within the body but possesses several characteristic features. First is the absence of fenestrations within the endothelial cells; communication can only occur through the cellular cytoplasm directly or via second messenger, or by paracellular routes. However, this route is itself limited by the second major feature of BBB endothelial cells, the presence of an extensive junctional complex composed of tight (TJ) and adherens (AJ) junctions [3], that essentially prevent uncontrolled cellular and molecular ingress into the brain [4][5]. Uptake of necessary nutrients into the brain is rather actively governed by the wide array of highly efficient influx and efflux transporters found within the endothelium, together acting to permit selective nutrient uptake and to actively remove metabolic waste products [6].

The endothelium, important as it is as the primary site of expression of TJ complexes within the BBB, is supported by numerous other cellular and non-cellular elements. Immediately adjacent to the endothelial cells is a complex basement lamina formed of four major glycoprotein family members, laminins, collagen IV isoforms, heparin sulphate proteoglycans and nidogens [7]. This basement lamina is actually composed of a pair of adjacent protein layers, produced by the endothelial cells and by perivascular astrocytes respectively, that whilst separate in larger vessels cannot be structurally distinguished at the level of the capillaries. The laminae can be discriminated by laminin complement however, with the endothelial basement layer containing laminin-411 and -511, whilst that derived from astrocytes is composed of laminin-111 and -211 [8]. This basement lamina is a functional as well as structural component of the BBB, being actively involved in communication and transport from the circulation to the neural parenchyma [9], and in the maintenance of TJ-mediated barrier integrity [10][11].

Two major cell types are found within the basement lamina, perivascular macrophages and pericytes, both of which play important albeit quite different roles in governing the BBB and its behaviour. Perivascular macrophages are the primary agents of immunosurveillance within the cerebral vasculature [12], but also facilitate glymphatic and intramural fluid drainage from the brain parenchyma to the circulation [13][14]. Interestingly there is also evidence that they may partially replace barrier function in the brain regions such as the area postrema that lack inter-endothelial TJs [15]. Pericytes in turn

play a number of important roles within the BBB, including governing capillary diameter and hence cerebral blood flow distribution [16][17], regulating angiogenesis within the brain [18] and directly contributing to BBB integrity through modification of TJs [19][20].

The final major component of the BBB are the perivascular astrocytes, found on the parenchymal side of the basement lamina, which respond to pericytes-derived cues by fully enveloping blood vessels with extended processes, the so-called astrocyte end-feet [21]. These processes provide dynamic structural support to the BBB, both through production of the laminins that form a key part of the basement lamina [8], and through active promotion of inter-endothelial cell TJ formation [22][23]. Beyond this structural support, astrocytes functionally contribute to the regulation of substrate transport from the blood to the brain parenchyma and vice versa, actively taking up water through the channel aquaporin-4 [24], nutrients through a broad complement of nutrient transporters [23] and removing neuronal metabolic waste from the brain tissue to the blood for renal or hepatic clearance [25][26].

Together, these diverse structural and functional elements of the cerebral vasculature form the BBB and endow it with an extraordinarily strong barrier function. Free diffusion of all but the smallest molecules between the vasculature and the brain parenchyma is essentially prevented, allowing for tight homeostatic control of the brain's micro-environment, and incidentally offering the opportunity to experimentally study BBB permeability through administration of different molecular weight tracers [27]. Despite this strength, the BBB is not a static structure but is rather highly plastic in response to challenge and demand with microbe-derived influences, among others, being powerful modulators of its function.

3. Regulation of BBB Integrity by Microbial Metabolites

Similarly to the gut epithelium, there are two major pathways by which microbial elements can affect the BBB in the absence of overt disease, either through the actions of microbial structural components or through those of microbe-derived metabolite. Of these, the effects of microbial components has received the greatest attention, with a substantial body of support having built up indicating that these agents can directly and indirectly regulate BBB integrity and thereby profoundly affect communication between the circulation and the brain.

That the BBB can be so targeted has been reported since the late 1950's, with studies showing injection of rabbits with LPS to rapidly but temporarily increase access to the brain for co-administered tracers [28], with LPS treatment since becoming one of the most widely-used experimental models of BBB damage, despite its pleiotropic effects on the body and thus difficulty in interpreting exactly how it works. LPS has been shown to affect BBB integrity in several ways, including by modulating absorptive transcytosis [29], promoting immune cell adhesion and trafficking [30], and modifying expression of major efflux transporter systems such as P-glycoprotein [31][32]. Beyond these functional changes to the BBB, LPS can also directly disrupt cell–cell junctional complexes in the cerebrovascular endothelium, reducing expression of TJ components and JAMs [33][34][35]. The exact mechanism(s) underlying these effects of LPS remain uncertain, with evidence indicating roles for the CD14–TLR4 complex itself [36], MAP kinase-driven activation of matrix metalloproteases [37], stimulation of NADPH oxidase and production of reactive oxygen species [38], and indirect effects caused via systemic cytokine production [39]. Notably however, the increase in BBB permeability induced by acute LPS treatment is relatively short-lived [40], prompting the interpretation that changes in BBB function may be part of the adaptive response to inflammation/infection, and may be a trigger for physiological sickness behaviour and fever [41].

As with the intestinal epithelium and the epidermis, the first evidence that the BBB is a target for the actions of microbe-derived metabolites came from analysis of germ-free mice [42]. Development and maturation of the BBB was markedly compromised in these animals, with enhanced permeability to protein tracers apparent in both embryos and adults. Whilst vascular density and pericyte coverage was unaltered, germ-free mice showed significant TJ disruption, with reduced expression and altered localisation of both claudin-5 and occludin, though not ZO-1 in all brain regions examined. Supporting these findings, similar disruption in hippocampal expression of claudin-5 and occludin was seen in mice fed with non-adsorbed, broad-spectrum antibiotics [43][44]. Importantly, BBB disruption was ameliorated upon either colonisation of germ-free mice with a conventional murine microbiota, with either of two SCFA-producing bacterial strains, or upon feeding with a sodium butyrate solution [42], strongly implicating SCFAs as the principal mediating factor, akin to their actions upon intestinal epithelial TJs.

The idea that butyrate is beneficial is further supported by work showing that administration of high concentrations of the SCFA to protect against BBB damage in vivo limits both Evans blue tracer extravasation into the parenchyma and brain oedema in rodent models of traumatic brain injury [45] and ischaemic injury [46], in both cases providing notable protection when administered post-injury. Moreover, while analysis was not made of TJ molecules in the ischaemia study, the brain

capillaries of mice that had received traumatic brain injury expressed markedly lower levels of occludin and ZO-1, changes which were significantly ameliorated by post-injury butyrate treatment [45].

The protective effects of butyrate in these studies were largely attributed to its role as an HDAC inhibitor, but there is evidence that SCFAs may protect at lower concentrations through their signalling at the G protein-coupled receptors FFAR2 and FFAR3. Administration of physiologically relevant SCFA concentrations prevented disruption to TJ structure and hence barrier permeability through down-regulation of the LPS co-receptor CD14 and activation of the antioxidant master regulatory transcription factor Nrf2, again reinforcing the idea of a protective role for SCFAs. These effects of SCFAs have since been extended in the identification of downstream regulation by butyrate/propionate of cytoskeletal components and TJ localisation [47].

While most studies have focussed on the role of SCFAs, they are not the only class of microbe-derived molecule that are active at the BBB, with evidence suggesting that bile acids, methylamines and p-cresol conjugates are capable of influencing barrier permeability in vitro and in vivo. Bile acids are critically required for dietary lipid solubilisation and uptake [48], and are classed as either primary, produced by hepatic cholesterol metabolism, or secondary, where primary acids have undergone further metabolism by enteric microbes. Members of both classes of bile acid have been shown to damage BBB function, at both very high [49], and more physiologically relevant concentrations [50]. The primary chenodeoxycholic acid and the secondary deoxycholic acid both increased the permeability of the rat BBB in vivo and disrupted the expression pattern of occludin, ZO-1 and ZO-2 in rat brain microvascular endothelial cells in vitro [50]. Interestingly, this disruption was not due to changes in expression of protein or mRNA expression for these molecules but was rather driven by enhanced phosphorylation of occludin. In contrast, human brain microvascular endothelial cells treated in vitro with the secondary bile acid ursodeoxycholic acid were protected against bilirubin-induced permeability damage [51], suggesting that bile acid treatment is not purely negative. Further studies into the role(s) played by bile acids in governing BBB integrity are clearly warranted.

Several microbe-derived metabolites have been found to affect cardiovascular function, most prominently the dietary methylamines, trimethylamine (TMA) and trimethylamine N-oxide (TMAO). Levels of TMAO, derived by microbial processing of choline and L-carnitine to TMA and its subsequent oxidation in the liver, have been correlated with cardiovascular disease in numerous population-level studies (reviewed in [52]). Importantly though, not all population studies have replicated these links [53][54] and TMAO is protective in animal models of atherosclerosis [55], hypertension [56], non-alcoholic steatohepatitis [57] and impaired glucose tolerance [58]. In light of these discrepancies, researchers compared the effects of TMA and TMAO upon the BBB. Notably, researchers found marked differences between the effects of TMA and TMAO upon an in vitro model of the BBB endothelium, with TMA significantly enhancing endothelial permeability via disruption of both the actin cytoskeleton and ZO-1 distribution, indicative of damage to TJ complexes [59]. In contrast, TMAO enhanced both the cortical distribution of actin and ZO-1, acting through the mobilisation of annexin A1, a key TJ regulatory protein [60], leading to a greater permeability barrier. These effects of TMAO have been replicated in vivo, where pre-treatment of mice with methylamine protected BBB integrity in the face of both acute and chronic inflammatory challenge, effectively preserving cognitive function [59]. That the relatively beneficial TMAO is a host metabolic derivative of microbe-produced and considerably more detrimental TMA highlights the role of host processes in detoxifying potentially damaging metabolites.

Further evidence for the modulatory influence of host enzymes upon microbial metabolite effect comes from the study of *p*-cresol conjugates. Primarily, *p*-cresol is produced by microbial degradation of the aromatic amino acids tyrosine and phenylalanine within the gut, whereupon it crosses the intestinal wall into the portal vasculature [61]. Very little native *p*-cresol is found in the systemic circulation, rather it is rapidly and almost completely conjugated by host hepatic and enteric enzymes into *p*-cresol sulfate (pCS) and *p*-cresol glucuronide (pCG) [62] at a ratio of approximately 9:1 in humans or 1:1 in mice [63]. Interestingly, although both these conjugates can affect the BBB, their effects in vivo are essentially opposite in nature. The studies of pCS identified potent permeabilising effects of this metabolite upon the BBB, acting through stimulation of the EGF receptor to trigger mobilisation of matrix metalloproteinases-2 and -9, damaging BBB integrity and inducing vascular leakage of macromolecules into the brain parenchyma [64]. In contrast, pCG had limited direct effects upon the BBB, but was able to almost completely prevent the permeabilising effects of either exogenous or circulating LPS in vitro and in vivo, acting through antagonism at the TLR4 receptor complex [65]. It seems highly likely that other such interactions exist between different host processing enzymes, microbial metabolites and/or microbial structural components, both at the BBB and other physiological barrier systems, indicating a vast scope for investigation and discovery.

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