Immunity
Review

The Gut Immune Barrier and the Blood-Brain Barrier: Are They So Different?

Richard Daneman[1,*] and Maria Rescigno[2,*]
[1]University of California, San Francisco, Department of Anatomy, San Francisco, CA 94143-0452, USA
[2]European Institute of Oncology, Department of experimental Oncology, 20139 Milan, Italy
*Correspondence: richard.daneman@ucsf.edu (R.D.), maria.rescigno@ifom-ieo-campus.it (M.R.)
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In order to protect itself from a diverse set of environmental pathogens and toxins, the body has developed a number of barrier mechanisms to limit the entry of potential hazards. Here, we compare two such barriers: the gut immune barrier, which is the primary barrier against pathogens and toxins ingested in food, and the blood-brain barrier, which protects the central nervous system from pathogens and toxins in the blood. Although each barrier provides defense in very different environments, there are many similarities in their mechanisms of action. In both cases, there is a physical barrier formed by a cellular layer that tightly regulates the movement of ions, molecules, and cells between two tissue spaces. These barrier cells interact with different cell types, which dynamically regulate their function, and with a different array of immune cells that survey the physical barrier and provide innate and adaptive immunity.

Introduction

The gut immune barrier (GIB) is the first line of defense against any potential harmful agents that have been ingested in food. The GIB has to constantly deal with innocuous food antigens and with the microbial flora (collectively called the microbiota) that are required for digestion and for conferring an initial protection against invading pathogens (Ley et al., 2006). Thus, the GIB is equipped to interact with and tolerate the microbiota, induce systemic tolerance to food antigens, and fight any possible invader. Defects in these functions can lead to intestinal disorders such as inflammatory bowel disease and irritable bowel syndrome, food allergy or intolerance, and microbial infection.

The blood-brain barrier (BBB) is a specialized structure formed by the blood vessels of the central nervous system (CNS). In most tissues, blood vessels are leaky, allowing a relatively free flow of molecules and ions from the blood into the tissue; however, in the CNS, the blood vessels tightly restrict the flow of blood-borne ions, molecules, and cells from entering the neural tissue (Gloor et al., 2001; Rubin and Staddon, 1999; Zlokovic, 2008). This BBB is a critical secondary barrier to protect CNS tissue, which fails to regenerate after injury and disease. Breakdown of the barrier occurs during many different neurological diseases, including stroke, multiple sclerosis (MS), and trauma and is an important component of the pathologies of these disorders (Zlokovic, 2008). Evasion of the barrier by pathogens and infection of the CNS can lead to potentially fatal neuroinflammatory diseases including meningitis, encephalitis, or focal abscesses. These infections can cause severe damage to the CNS, which can lead to paralysis, dementia, and death. In fact, bacterial meningitis is a top ten leading cause of bacterial deaths in the world (Kim, 2008).

For each barrier, the body has therefore evolved a complex set of cellular and molecular mechanisms to protect the organism from toxins and infections. In each case, there is a physical barrier formed by a cellular layer that tightly regulates the movement of ions, molecules, and cells between two tissue spaces. For the GIB, this consists of gut epithelial cells, separating the gut lumen from the internal space, whereas for the BBB, this consists of CNS endothelial cells, which separate the lumen of blood vessels from the CNS parenchyma. These barrier cells are not isolated but interact with different cell types, which dynamically regulate their function. For the GIB, the epithelial cells are associated with enterochromaffin cells, goblet cells, and Paneth cells and are in close contact with enteric glial cells (Figure 1). For the BBB capillaries, pericytes are situated on the abulimnal surface of the endothelial tube, and this blood vessel is surrounded by a basal lamina. Neural cells form intimate contacts with the vessels, most noteably astrocytes, a glial cell that extends long processes whose endfeet ensheath the vessels (Figure 1). Each barrier also contains a different array of immune cells that survey the physical barrier and provide innate and adaptive immunity. Here, we compare and contrast the different properties of the gut immune barrier and the blood-brain barrier as well as the mechanisms that specific pathogens have developed to evade them.

Physical Barrier: Mucous Layer and Glycocalyx

An important difference between the GIB and the BBB is the microenvironment to which the two barriers are exposed. Whereas the BBB is only occasionally exposed to microorganisms, the GIB is constantly in contact with the microbiota, much of which is beneficial to digestion and protection. Thus, an important difference between the two barriers is that the GIB is equipped with a mucous layer that forms a first layer of protection between the gut and the external world by physically separating the microbiota from the epithelial barrier.

The mucus layer is like a mesh of networking fibers made primarily of mucins, glycoproteins, and lipids that allows the passage of definite-size molecules (Neutra et al., 1996) and excludes bacteria from contacting the epithelial barrier. It is

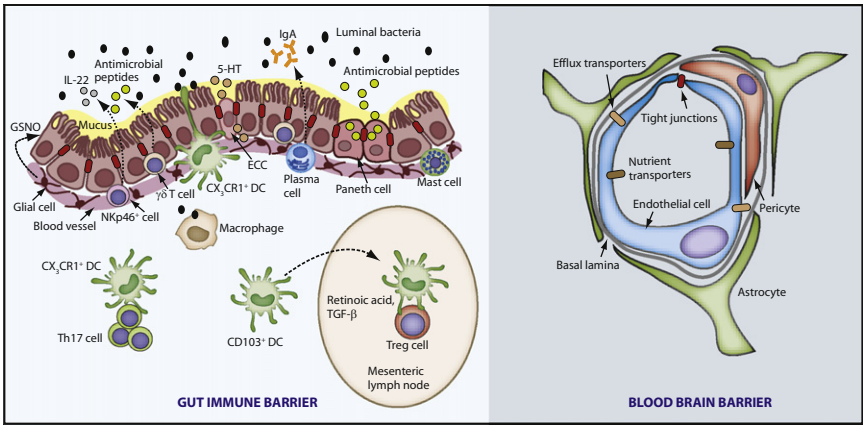

Figure 1. The Gut Immune and Blood-Brain Barrier

The GIB is composed of a mucus layer and a physical barrier. This is composed by epithelial cells, enterochromaffin cells (ECCs), and Paneth cells. ECCs release neuroimmunomodulatory mediators such as serotonin (5-HT). Paneth cells release antimicrobial peptides. A rim of tight junctions seals the epithelial barrier and controls the paracellular route of solutes, nutrients, and ions. Within the epithelial barrier, immune cells such as intraepithelial lymphocytes (primarily γδT cells) and CX_3CR1^+ dendritic cells (DCs) are scattered. γδ T cells release antimicrobial peptides after epithelial barrier disruption, whereas DCs can actively participate in bacterial handling and activate Th17 cells. DCs have also been shown to enter the luminal side after bacterial infection. Below the basal membrane, blood vessels are found that are ensheathed by glial cells. The latter are also in contact with epithelial cells and preserve barrier integrity via the release of S-nitrosoglutathione (GSNO). In the lamina propria (LP), other immune cells are found, such as $NKp46^+RORγt$ cells, that release IL-22 in response to bacteria and macrophages that are "inflammatory anergic" but that can still kill bacteria. Another population of $CD103^+$ DCs can also be found in the LP that induces the differentiation of Treg cells after migration to the mesenteric lymph nodes (MLN). Plasma cells (PCs) can release IgA that can serve for immune exclusion and anchorage of commensal bacteria to mucus. Mast cells are a link between the gut immune system and the brain as they can respond to and release neurotransmitters and immune mediators. In contrast, the properties of the BBB are manifested by CNS endothelial cells (blue), which form the walls of the blood vessels. These cells are held together by tight junctions creating a paracellular barrier and lack transcytotic vesicles, creating a transcellular barrier. These cells also express a series of transporters, to both efflux a variety of lipophillic molecules and to deliver specific nutrients to the CNS. The vascular endothelial cells are associated with pericytes (red), which together are surrounded by a basal lamina. In addition, different immune cells, including perivascular macrophages and mast cells, are associated with the vessels (not depicted). Astrocytes send out processes whose endfeet (green) ensheath the vessels. Interactions between endothelial cells and astrocytes, pericytes, and other neural and immune cells regulate the properties of the BBB.

composed of two fractions: a viscous, loose luminal gel and an epithelium-adherent firm fraction that are extensively reviewed elsewhere (Kelly et al., 2007; Linden et al., 2008; Macfarlane and Dillon, 2007; Sonnenburg et al., 2004). The firm mucous layer constitutes the epithelial cell-associated glycocalyx where mucins are found in their membrane-bound form. In the mouse, this capacity is dependent on the expression of mucin-2 (muc)2 (Johansson et al., 2008). The loose mucous layer is formed by soluble mucins that are released primarily by goblet cells that are interspersed between epithelial cells (Linden et al., 2008). This layer is exploited by the microorganisms to form a biofilm that allows their growth and prevents their washout with the luminal content, due to intestinal peristalsis (Macfarlane and Dillon, 2007). Some bacterial species digest mucous components and can be found also embedded in the mucus. The thickness and composition of the mucous layer vary between the different portions of the gastrointestinal tract (Atuma et al., 2001; Robbe et al., 2004) and is dependent on the density of the microbiota, being thicker where the microbiota is more abundant. Hence, the mucous layer serves the dual function of physically separating the bacteria from the epithelial barrier and of providing an anchorage system for their growth.

Although the BBB does not contain a mucous layer per se, the luminal surface of the endothelial cells is covered by a complex glycocalyx, a dense layer of negatively charged carbohydrates (Van Teeffelen et al., 2007). The glycocalyx lines the vascular wall in all tissues, and it remains to be determined whether the glycocalyx at the BBB has specific properties to protect the CNS. The vascular glycocalyx has been shown to regulate vascular homeostasis, protect the vascular wall from shear stress, and modulate its interaction with blood cells (Van Teeffelen et al., 2007). Furthermore, the glycocalyx has been proposed to act as a molecular sieve to block interaction of large blood-borne molecules with the vascular wall (Vink and Duling, 1996, 2000). As with the mucus layer, specific pathogens including *Haemophilus somnus*, can bind to the glycocalyx and use it to anchor to CNS endothelial cells (Behling-Kelly et al., 2006).

Cellular Barrier

Each barrier not only provides protection from invading pathogens, but is also important to control the microenvironment of the tissue and, therefore, tightly regulates the movement of molecules and ions between the cellular spaces. For the gut, this is important to regulate nutrients, water, and salt absorption and resorption into the digestive tract. For the CNS, this is important because neural activity relies on precise cellular ion concentrations and the BBB separates the neural tissue from the ionic fluctuations in the blood. Crucial to this tight regulation are the barrier properties of the epithelial and endothelial cell layers.

A fundamental difference between the GIB and the BBB is the origin of the cell types that form the barrier. In the gut, the epithelial cells are endodermal in origin, whereas in the CNS, the endothelial cells are mesodermal in origin. This difference stems from the fact that gut epithelia protects organisms from external hazards, whereas the BBB protects the CNS from hazards that are within the organism. Despite this difference, a striking observation is that CNS endothelial cells, but not endothelial cells in non-neural tissues, resemble epithelial cells in that they are held together by tight junctions that polarize the cells to form distinct apical and basolateral membrane compartments (Zlokovic, 2008). Interestingly, mesodermal-to-epithelial cellular transitions, and vice versa, are important events that occur during development of different tissues and also the pathogenesis of cancer (Acloque et al., 2009).

Paracellular Junctions

Gut epithelial cells are sealed with each other by a circular rim of tight junctions (TJ). TJs are formed by integral membrane

proteins called Occludin, Junctional adhesion molecule (JAM), and Claudins and by cytoplasmic proteins connecting the TJ with the cytoskeleton (Zonula occludens [ZO]-1, ZO-2, ZO-3, 7H6, and Cingulin). TJs do not form impermeable seals but control the paracellular route of solutes by allowing the absorption of essential nutrients via the formation of a dynamic structure that is modulated by pharmacological, physiological, and pathological stimuli (reviewed in Shen and Turner, 2006). Mutagenesis studies of Claudin family molecules suggest that specific amino acid residues in the first extracellular domain of these tetraspanins determines the size and charge selectivity of tight junction pores (Colegio et al., 2003). In the GIB, Claudin 1, -4, -5, -7, and -8 are most represented (Zeissig et al., 2007). TJs are not static under steady state and the proteins are continuously displaced and substituted with different kinetics according to the analyzed TJ protein and physiological setting (Shen et al., 2008).

Endothelial cells in the brain, unlike endothelial cells in other tissues, are coupled by ultrastructural tight junctions (Ballabh et al., 2004; Gloor et al., 2001; Rubin and Staddon, 1999). The molecular composition of BBB endothelial tight junctions shows remarkable similarities to those in epithelial cells in that they are formed by strands of Occludin, JAM, and Claudin molecules that are linked to the cytoskeleton by Zonula Occludens (Hirase et al., 1997; Morita et al., 1999; Nitta et al., 2003). Claudin 5 is critical to BBB formation, as Claudin 5-deficient mice show a size selective leakage of the BBB, whereas Occludin may specifically regulate calcium flux between the blood and the brain because Occludin deficient mice still form high-resistance junctions but have calcification of the CNS (Nitta et al., 2003; Saitou et al., 2000). Claudin 1, -3, and -12 have also been identified at the BBB (Liebner et al., 2008; Mark and Davis, 2002; Nitta et al., 2003; Wolburg et al., 2003).

The tight junctions that form the paracellular barrier at the GIB and BBB display remarkable molecular similarities, and the specific claudin family members may be important for determining the permeability in each tissue. Moreover, downregulation or redistribution of TJ proteins are observed under pathological conditions in which the barriers are disrupted, including inflammatory bowel disease (IBD) (Turksen and Troy, 2004; Zeissig et al., 2007) and irritable bowel syndrome (IBS) (Dunlop et al., 2006; Spiller, 2008) for the GIB, and stroke (Sandoval and Witt, 2008) and MS (Correale and Villa, 2007) for the BBB.

Transcellular Barriers

The epithelial and endothelial cells also contain barrier mechanisms that regulate the movement of molecules and ions through the cell layers. Although the paraceullular barrier properties of the GIB are quite conserved throughout the whole intestine, transcellular barrier properties of the GIB differ according to the section of the intestine that is considered. For instance, the small intestine is primarily devoted to the degradation and absorption of food. This is achieved via the vagination of the mucosa to form villi that increase the surface of exposure to nutrients. In addition, the apical face of small intestinal epithelial cells forms a brush border consisting of microvilli that are specialized for the digestion and absorption function and further increases the absorption area. Food antigens are internalized principally via endocytosis (Weiner, 1988), and nutrient sensing results in the activation of the enteric neuronal pathways and the regulation of food intake (Raybould, 2008). The large intestine is involved in the absorption of water and salts and in the digestion of complex molecules (that is accomplished via the symbiosis with the microbiota). For this reason, as mentioned above, the colon GIB is equipped with a thicker layer of mucus (Atuma et al., 2001; Robbe et al., 2004). It is important to mention that specialized epithelial cells, called M cells, can also be found in the epithelium of the small and large intestine (Jang et al., 2004). These cells lack an organized brush border and are more easily approached by bacteria and toxins. A possible role of M cells in the internalization of food antigens remains to be established.

CNS endothelial cells contain a variety of specialized properties to tightly regulate transcellular passage of molecules and ion. CNS endothelial cells contain fewer transcytotic vesicles than endothelial cells in other tissues, thus limiting the transcellular passage of hydrophilic molecules and ions from the blood to the brain (Ballabh et al., 2004; Gloor et al., 2001; Rubin and Staddon, 1999). In addition, CNS endothelial cells express a series of efflux transporters, including P-glycoprotein, which are able to transport a variety of lipophilic molecules that are able to passively diffuse from the blood into the brain, through the endothelial lipid membranes, and back to the blood (Schinkel et al., 1994; Schinkel et al., 1996). Whereas these properties allow CNS endothelial cells to form a transcellular barrier, these cells also express a variety of transporters to deliver specific nutrients into the brain, including glucose, amino acids, and vitamins (Zlokovic, 2008). Thus, CNS endothelial cells are simultaneously able to exclude potential toxins and deliver specific nutrients into the neural tissue. For both barriers, the combination of paracellular tight junctions and transcellular transport properties allow the cell layers to tightly regulate the movement of molecules and ions between the two tissue spaces.

Regulation of the Barrier Properties

Another important similarity between the GIB and the BBB is that barrier properties are regulated by interactions with other cells. Although the properties of the BBB are manifested in the CNS endothelial cells, transplantation studies have demonstrated that they are not intrinsic to endothelial cells but induced by interactions with neural tissue (Stewart and Wiley, 1981). Due to the close association between astrocytes and endothelial cells, extensive studies have examined the role of astrocytes in regulating the properties of the BBB. Astrocyte feeder layers increase the transcellular electrical resistance of endothelial monolayers in vitro (Dehouck et al., 1990; Hayashi et al., 1997; Isobe et al., 1996), and transplanted astrocytes are sufficient to induce barrier properties in non-neural endothelial cells in vivo (Janzer and Raff, 1987). Several molecules have been implicated in mediating this response, including astrocyte src kinase SSeCKs and secreted Angiotensin II (Lee et al., 2003; Wosik et al., 2007). These studies have led to a two-step model for BBB formation in which angiogenesis in the CNS proceeds by the same mechanism as angiogenesis in other tissues, with the formation of leaky vessels, which are then induced to form the BBB by interactions with astrocytes. Several studies, however, have identified that BBB properties in CNS endothelial cells are present during development prior to astrocyte generation (Bauer et al., 1993; Ek et al., 2006), and in vitro studies have implicated both neural

stem cells and pericytes in regulating barrier functions of CNS endothelial cells (Dohgu et al., 2005; Hori et al., 2004; Weidenfeller et al., 2007). Moreover, recent work has demonstrated that neural stem cell-derived Wnt-β-catenin signaling is required for CNS angiogenesis, but not angiogenesis in non-neural tissue, and also induces BBB-specific transporter expression and tight junction formation (Daneman et al., 2009; Liebner et al., 2008; Stenman et al., 2008). This suggests that angiogenesis and BBB induction may be tightly linked. If BBB properties are induced during angiogenesis, what role do astrocytes play in regulating BBB function? To answer this question it is important to identify whether barrier properties in CNS endothelial cells are induced by a single induction event during development or need to be maintained by constant signaling throughout life. Evidence suggests that BBB regulation may involve a complex combination of both mechanisms. For instance, when purified CNS endothelial cells are grown in culture without neural tissue, they express many BBB-specific genes, including molecular transporters and tight junction molecules, but fail to form high-resistance junctions and lack polarity (Rubin et al., 1991; Weidenfeller et al., 2007). These results suggest that certain aspects of BBB-specific gene expression may be induced during angiogenesis, but function of the barrier may need to be regulated dynamically throughout life by interaction with astrocytes as well as pericytes and different neural and immune cells.

For the GIB, enteric glial cells that are morphologically very similar to astrocytes, with which they share many common markers and functions, dynamically regulate the permeability of the barrier. Besides protecting the enteric neurons, glial cells participate to preserve intestinal epithelial barrier integrity. In analogy with astrocytes in BBB function, enteric glial cells, whose projections come very close to epithelial cells (<1 μm) (Neunlist et al., 2007) and blood capillaries (Hanani and Reichenbach, 1994), release soluble factors that control paracellular permeability via a direct action on TJ proteins (Neunlist et al., 2008). One of these factors is S-nitrosoglutathione (Savidge et al., 2007). Conditional ablation of enteric glial cells results in an increase in vascular and paracellular permeability that leads to lethal intestinal disease (Bush et al., 1998; Cornet et al., 2001). Concomitant administration of S-nitrosoglutathione during enteric glial cell ablation inhibits the increased intestinal epithelial barrier permeability and protects mice from colitis (Savidge et al., 2007). Restored barrier function is associated with an increase of TJ protein Occludin and ZO-1 expression (Savidge et al., 2007). Hence, glial cells play a fundamental role in intestinal epithelial barrier integrity.

The GIB epithelial barrier also contains enterochromaffin cells that represent the most abundant neuroendocrine cells in the gut. These cells have a triangular shape and are interspersed among epithelial cells. They are considered to be the first "sensors" of the luminal content, which they reach with a very thin luminal extension. Luminal stimuli activate enterochromaffin cells to release serotonin (5-hydroxytryptamine, 5-HT) (Spiller, 2008). Ninety-five percent of all gut serotonin is produced by enterochromaffin cells. Serotonin has a variety of targets, such as neighboring epithelial cells, neuronal afferents in the lamina propria, and immune cells. The outcome is activation of gut contractility and motility, leading to diarrhea and vomiting, which are primitive mechanisms of protection against infection. During infection, the number of enterochromaffin cells increases via a T cell-dependent mechanism (Wang et al., 2007). Th2, but not Th1, T cells are involved in enterochromaffin cell hyperplasia (Motomura et al., 2008). Serotonin has been shown to be involved also in the control of epithelial permeability, suggesting that enterochromaffin cells may participate in controlling GIB permeability (Yamada et al., 2003).

Further research identifying the molecules that regulate each barrier during health and disease will increase our understanding of the similarities and differences between barriers throughout the body.

Immune Barrier

Each barrier is important to regulate tissue immunity for two reasons. First, each cellular barrier regulates the movement of immune cells in order to maintain the appropriate contingent in each separated tissue space in order to fight infections but limit potentially damaging inflammation. Second, in both cases, immune cells are associated with the physical barrier to provide immunity at entry sites. The GIB epithelial cells are associated with intraepithelial lymphocytes and dendritic cells (DCs), whereas BBB endothelial cells are associated with perivascular macrophages and mast cells (Ibrahim et al., 1980; Williams et al., 2001). Both barriers are reinforced by immune cells that are located just underneath the physical barrier. The lamina propria of the gut contains lymphocytes, plasma cells, DCs, macrophages, mast cells, and NK cells, whereas the CNS contains primarily microglial cells (Streit et al., 2005). The different immune cell composition leads to different adaptive immune responses between the two tissues.

Regulation of Immune Cell Migration

Both barriers regulate the movement of immune cells between two tissue spaces; however, there is a difference in the directionality of this regulation. For the GIB, immune cells originate from the abluminal tissue (lamina propria) and their movement into the gut lumen is regulated. For the BBB, the immune cells originate in the luminal space (blood) and their movement is regulated into the abluminal CNS tissue. One important similarity is that under steady-state the two barriers allow only very limited passage of immune cells for different reasons. The GIB has to preserve the homeostasis of the microbiota and limits its encounter with immune cells, whereas the BBB has to preserve the homeostasis of the brain and limits the passage of immune cells, especially lymphocytes, leading to minimal immune surveillance of the CNS. In both cases, massive infiltration of immune cells is observed in pathological conditions.

The CNS has been considered an "immune-privileged site" due to a lack of graft rejection when tissue is transplanted into the CNS. This "privilege" is due to a lack of draining lymphatics, specific properties of local antigen presenting cells, and an almost complete lack of lymphocytes, which are impeded entry by the BBB (Carson et al., 2006). Understanding the entry of immune cells across barriers is important not only for understanding tissue immune function, but also understanding how pathogens cross barriers because bacteria, fungi, and viruses can cross the BBB either by mimicking immune cells or by infecting migrating immune cells. For vasculature, leukocyte migration through blood vessels involves a rolling adhesion to

the endothelium, tight adhesion for a high-affinity interaction, and finally, migration either between endothelial cells or through endothelial vesicular compartments. In general, weak binding is initiated between endothelial selectin molecules and leuckocyte carbohydrates, and tight adhesions are solidified through binding of leukocyte integrins with endothelial cell immunoglobin superfamily members such as ICAM1 and VCAM (Huang et al., 2006). P-selectin and ICAM1 exhibit lower resting expression in brain vessels than peripheral vessels, which may account for the low level of CNS immune surveillance (Aird, 2007; Henninger et al., 1997). These molecules, however, have been shown to be upregulated during pathological conditions, including infection, autoimmune disease, or stroke (Engelhardt, 2008; Huang et al., 2006). In addition, passive transfer of T cells activated in vitro, but not resting T cells, can cross an intact BBB, suggesting that immune surveillance can occur in healthy individuals (Engelhardt, 2006; Hickey et al., 1991). These results suggest that T cells activated outside the immune system can cross the resting BBB, but unactivated T cells can only enter the CNS during neuroinflammatory process such as those that occur in patients with MS.

Recently, the gut has also been regarded as an ''immune-privileged site,'' but not in the conventional sense. This is due to the necessity of the immune system to avoid inflammation in response to microbial-associated inflammatory stimuli and to induce tolerance toward food antigens (Iweala and Nagler, 2006). Unlike the BBB, in the gut, immune privilege is not intended to control the entrance of potentially autoreactive adaptive immune cells. In contrast, adaptive immune cells, primarily T regulatory cells, IgA-secreting plasma cells, $\gamma\delta$ T cells, and Th17 cells, are present also at steady state and participate to preserve gut homeostasis. T regulatory cells protect the host from tissue damage due to microbial-derived inflammation and mediate food tolerance (Belkaid and Tarbell, 2009). Th17 cells and $\gamma\delta$ T cells instead offer a first line of protection toward commensal bacteria and, together with NKp46$^+$ cells, allow epithelial cell repair (see below). In order to be recruited into the intestine, lymphocytes express gut homing receptors that are imprinted during their activation by mucosal DCs (Mora et al., 2008). A different combination of gut-homing receptors is required to home to the small or large intestine: coexpression of $\alpha4\beta7$ and CCR9 allows recruitment into the small intestine, whereas CCR10 directs lymphocytes to the large intestines (Macpherson et al., 2008).

Gut resident immune cells are set to minimize microbial-derived inflammation and participate in epithelial repair, but still retain their microbicidal activity. Further, in the gut, it is necessary to discriminate between dangerous and harmless microorganisms. This control seems to be carried out primarily by epithelial cells for their capacity to distinguish between invasive and noninvasive bacteria. This is due partly to the polarized expression of pattern recognition receptors that recognize common microbial structures either on the basolateral membrane or intracellularly, and partly to the differential response obtained when apical or basolateral receptors are engaged (Lee et al., 2006; Rescigno et al., 2008). As long as the bacteria are kept outside the physical barrier, there is no initiation of inflammation. However, if a bug succeeds to cross the epithelial cells (a characteristic normally associated with pathogenicity), a danger signal is released by the epithelial cells that leads to the recruitment of inflammatory cells, including neutrophils and monocytes (Sansonetti, 2004), and to the induction of adaptive immunity. Recruited neutrophils (Chadwick et al., 1988) and, recently, also DCs, (Arques et al., 2009) are able to cross the epithelial barrier where they can potentially attack the bacteria and limit their crossing the epithelial barrier. Invasive bacteria are then handled by the resident immune cells and by the recruited phagocytes that recognize the invaders as potentially dangerous and proceed to their elimination. This is, however, associated with inflammation and tissue damage that, if protracted over time, can lead to chronic inflammation and the development of inflammatory disorders.

Barrier-Associated Immune Cells

GIB-associated immune cells (Figure 1) participate in the homeostasis of the gut to fight potential invaders but also to sense the external world. DCs, for instance, do not play simply a passive role, but actively participate in bacterial internalization across the epithelium and may serve as a tool to ''sense'' the external world (Rescigno et al., 2001). DCs express TJ proteins and can intercalate between epithelial cells for direct uptake of antigens across the intestinal lumen (Rescigno et al., 2001). These cells express CX3CR1 (Niess et al., 2005) and also have the capacity to induce Th17 cell type of responses (Atarashi et al., 2008; Denning et al., 2007). Th17 cells can confer protection toward a variety of bacteria and fungi (Dubin and Kolls, 2008). Another subset of lamina propria DCs expressing the CD103 marker are instead involved in driving the development of T regulatory cells (Sun et al., 2007). CD103$^+$ DCs cells are implicated in the development of tolerance to orally ingested antigens. Macrophages isolated from the lamina propria display reduced capacity to induce inflammation in response to microbial stimulation, but are fully capable of killing bacteria (reviewed in Kelsall, 2008). The intestinal barrier is also patrolled by intraepithelial T cells. The most abundant intestinal intraepithelial lymphocytes bear the $\gamma\delta$ T cell receptor (Kunisawa et al., 2007). $\gamma\delta$ T cells promote repair of injured gut epithelia (Komano et al., 1995). $\gamma\delta$ T cells play a major role in limiting the entrance of commensal bacteria after epithelial injury via the release of antimicrobial factors (Ismail et al., 2009). This response is mostly induced by the microbiota because in its absence (germ-free mice), the induction of the majority of genes related to inflammation and to the antimicrobial response is drastically reduced after intestinal epithelial disruption (Ismail et al., 2009).

Classical NK cells develop from hematopoietic stem cells in the bone marrow and thymus. These cells seed peripheral organs such as the spleen, lung, liver, and lymph nodes (Di Santo and Vosshenrich, 2006). Although NK cells have long been considered to be proinflammatory killer cells, recent evidence has shown the existence of subtypes of NK cells with distinct immunoregulatory function (Maroof et al., 2008). In the gut, one of these subsets (NKp46$^+$RORγt$^+$ cells) has been described that displays GIB protective function. These cells release IL-22 which is required both for epithelial cell repair and antibacterial activity (Cella et al., 2009; Luci et al., 2009; Sanos et al., 2009; Satoh-Takayama et al., 2008; Vivier et al., 2009; Zheng et al., 2008). Differently from classical NK cells, NKp46$^+$RORγt$^+$ cells are unable to release the cytokines IFN-γ, IL-12, or IL-18, thus

limiting the inflammatory reaction. Also, plasma cells are found in the lamina propria. These cells release, primarily, Immunoglobulin (Ig)-A that participates to mucosal defense as they immune-exclude IgA-coated bacteria (Cerutti and Rescigno, 2008). IgA can also serve to anchor commensal bacteria to the mucus and allow their colonization (Corthesy, 2007).

The GIB is also reinforced by a chemical component. This includes antimicrobial peptides such as defensins, angiogenins, defensins-like peptides that are released by enterocytes, or by Paneth cells that reside at the base of the crypts of Lieberkhun. The expression of antimicrobial peptides is regulated by the presence of indigenous microorganisms (Cash et al., 2006), via different mechanisms according to the class of peptides that are analyzed. The expression of α-defensins is controlled by the intracellular pattern recognition receptor belonging to the Nucleotide oligomerization domain (NOD)-2 (Kobayashi et al., 2005), whereas RegIIIγ, RegIIIγ, CRP-ductin, and RELMβ are regulated via the MyD88-dependent Toll-like receptor pathway (Vaishnava et al., 2008). Hence, commensals can regulate the host-microbial homeostasis at mucosal surfaces via the activation of pattern recognition receptors in epithelial and Paneth cells and the release of antimicrobial peptides. These peptides limit the translocation and propagation of microbes from mucosal surfaces to peripheral lymphoid tissues, thus actively participating to barrier function (Vaishnava et al., 2008).

At the BBB, perivascular macrophages reside on the parenchymal side of the endothelial cells between the vessel and astrocyte endfeet (Williams et al., 2001). These cells are derived from peripheral monocytes, and human transplantation studies have demonstrated that they are steadily replaced from hematogenous populations (Unger et al., 1993). Moreover, rodent bone marrow chimera studies have shown an 80% turnover of perivascular macrophages in 3 months (Vass et al., 1993), demonstrating a remarkable capability of these cells to cross an intact BBB. Perivascular macrophages have been shown to play a role in the innate immune response in that they phagocytose cellular debris and foreign particles (Williams et al., 2001). Studies have utilized intraventricular injection of mannosylated clodronate liposomes that completely deplete perivascular and meningeal macrophages from the CNS to study their role in infection. For pneumococcal meningitis infections, this resulted in an increased illness, higher bacterial loads in cerebrospinal fluid (CSF) and blood, and a decreased flux of leukocytes into CSF (Polfliet et al., 2001). In addition, perivascular macrophages can act as antigen-presenting cells (APCs) to initiate an adaptive immune response in certain pathological conditions (Hickey and Kimura, 1988). Taken together, these results suggest that perivascular macrophages play an important role in innate and adaptive immune response, and their perivascular localization provides an immune component at the BBB to further protect the CNS from invasion of pathogens. Although perivascular macrophages are an important element of the BBB, because of their frequent migration across the endothelial monolayer, these cells can also be harnessed to act as shuttles for pathogens to enter the CNS (Williams and Blakemore, 1990). This ''Trojan horse'' mechanism has been suggested for HIV and *Cryptococcus neoformans* infection of the CNS (Buckner et al., 2006; Charlier et al., 2009). For instance, in rodent infection studies, bone marrow-derived monocytes infected in vitro with *C. neoformans* had a 3.9-fold increased CNS infection rate compared to addition of free yeast (Charlier et al., 2009).

Microglial cells are monocyte-derived CNS resident cells that differ from perivascular macrophages in their localization, morphology, immunophenotype, and function. Microglial cell bodies are not intimately associated with vessels but are situated throughout the CNS parenchyma. These cells are highly ramified and contain cellular processes that touch the vasculature and can regulate BBB function. Microgliosis is a common feature of all CNS injury and disease and involves microglial cell division, hypertrophy, and changes in immunophenotype and secretory activity (Streit et al., 2005). Parabiosis studies have demonstrated that microglial cells are not recruited from hematogenous populations during microgliosis but through cell division within the CNS parenchyma (Ajami et al., 2007). Microglial cells act as a part of the innate immune response eliminating microorganisms and promoting wound healing by acting as tissue-specific phagocyte of foreign particles, macromolecules, and debris as well as producing growth factors and extracellular matrix (Streit et al., 2005). In addition, microglia can act as APCs to initiate an adaptive immune response. Therefore, microglia are CNS resident monocyte-derived cells that modulate immune responses in the CNS parenchyma.

Mast cells are present at both the GIB and the BBB, and they respond to and release mediators of both the nervous and immune system (Marshall, 2004). In the gut, mast cells are strategically located in proximity to blood vessels and enteric nerve endings (Stead et al., 1987) and are efficiently activated by a variety of mechanisms in response to bacterial or parasitic infection (Dawicki and Marshall, 2007). They release a series of mediators aimed at the recruitment of inflammatory cells that can have an important effect also on epithelial barrier permeability. Tryptase, for instance, can activate PAR-2 receptors on epithelial cells and increase epithelial permeability via modulation of TJ proteins (Cenac et al., 2004). PAR-2 receptors are also expressed on nerve endings, and their activation may result in neurogenic inflammation (Steinhoff et al., 2000). Not surprisingly, mast cell activation has been implicated in several stress-related intestinal disorders, like ulcerative colitis (UC) and IBS (Farhadi et al., 2007). Consistently, mice deficient in mast cells display increased anxiety-like behavior, but the effect on gut permeability or colitis has not been analyzed (Nautiyal et al., 2008). In the CNS, mast cells are associated with vessels in specific regions of the brains, most notably the dorsal thalamus and the circumventricular organs (Ibrahim et al., 1980). They have the ability to degranulate, and vesicular contents can be picked up by neurons (Wilhelm et al., 2005). However, the role of mast cells in nervous system development and function or immunity is not fully understood.

Intestinal Disorders: Inflammatory Bowel Disease and Irritable Bowel Syndrome

Given the important role of the GIB in controlling intestinal homeostasis defects linked to any of the components of GIB may participate to intestinal inflammatory disorders. IBD comprises UC and Crohn's disease (CD). CD is characterized by discontinuous inflammation that can affect the terminal ileum or the colon. The whole mucosal layers can be involved in the

disease, with 50% of patients displaying granuloma at histological examination. UC, by contrast, involves only the colon, and the inflammation is restricted to the superficial mucosa. These are multifactorial disorders with genetic and environmental components. The involvement of bacteria in IBD development has been demonstrated by the capacity of antibiotics to ameliorate the severity of the disease (Ewaschuk et al., 2006) and by the genetic association of IBD with mutations in the genes that encode CARD15 (NOD2) (Hampe et al., 2001; Hugot et al., 2001; Ogura et al., 2001), CARD4 (NOD1) (McGovern et al., 2005), or TLR4 (De Jager et al., 2007; Franchimont et al., 2004), which are sensors of bacteria, or mutations in autophagy-related genes that are important in innate defense (Hampe et al., 2007; Saitoh et al., 2008). Although long sought after, a clear association with any pathogenic bacteria has not, however, been demonstrated in the etiology of IBD. By contrast, IBD patients have been shown to immune-react to their autologous flora, whereas normal individuals react only to the heterologous flora (Duchmann et al., 1995). How these responses are initiated and whether there is a primary defect in intestinal permeability that allows entrance of otherwise excluded bacteria remains to be established. Also, as the noninflammatory phenotype of macrophages and DCs is conferred by the local microenvironment, defects in this compartment compromise the homeostasis of the gut via the inability of controlling the inflammatory potential of immune cells (Iliev et al., 2009a, 2009b; Rimoldi et al., 2005; Smythies et al., 2005). Another inflammatory disorder of the gut that can be associated to GIB dysfunction is IBS. IBS is a heterogeneous multifactorial disorder. IBS has been shown to be related to psychological stress, presumably via the release of corticotrophin-releasing hormone (CRH) (Fukudo, 2007). CRH can activate immune cells and induces mast cell degranulation and increased intestinal permeability (Wallon et al., 2008). This, together with the observation that the number of mast cells is increased in rectal biopsies from IBS patients independent of their IBS subtypes (Lee et al., 2008), links mast cell activation with IBS. It would be interesting to know whether defects associated with BBB permeability can participate in gastrointestinal disorders and vice-versa.

Neuroinflammation: Stroke and Multiple Sclerosis

Ischemic stroke is a debilitating disease resulting from the loss of blood flow to specific regions of the CNS. This results in breakdown of the BBB and includes infiltration of leukcoytes and both neutrophils and monocytes, but not lymphocytes, into the CNS (Huang et al., 2006). Insights into the cellular and molecular events that occur following stroke come from animal models of middle cerebral artery occlusion. After occlusion, secretion of the cytokines IL-1, IL-6, TNF-α, and TGF-β leads to an upregulation of ICAM-1 and Selectins on the vascular endothelium, which promotes leukcocyte migration across the BBB (Huang et al., 2006). This neuroinflammation is important for the severity of the disease because less damage is observed in P-selectin-deficient mice, ICAM1-deficient mice, or after delivery of antibodies against either molecule (Bowes et al., 1993; Connolly et al., 1996; Mayadas et al., 1993).

MS is a T cell-mediated autoimmune disease which targets myelin in the white matter of the CNS. In MS, lymphocytes enter the CNS, both because of focal disruptions of the BBB, and because of increased leuckoycte adhesion molecules on the inflamed BBB. In experimental autoimmune encephalitis (EAE), the mouse model of MS, lymphocyte integrin $\alpha_4\beta_1$ binding to endothelial VCAM1 is critical for recruitment of inflammatory cells to CNS (Baron et al., 1993; Yednock et al., 1992). Blocking this interaction with Natalizumab, an antibody against a4 integrin, has been beneficial for limiting the symptoms associated with MS (Polman et al., 2006). ICAM1 is also upregulated on CNS vessels during EAE, and ICAM1-deficient mice displayed reduced T cell infiltration and attenuated symptoms following EAE induction (Bullard et al., 2007; Steffen et al., 1994). In vitro coculture experiments suggest that T cell interactions with VCAM1 are required for firm adhesion to the endothelium and interactions with ICAM1 for transmigration (Engelhardt, 2006; Laschinger and Engelhardt, 2000; Lyck et al., 2003). In addition, the Ig superfamily adhesion molecule ALCAM is upregulated on CNS endothelial cells in MS lesions. ALCAM binds to CD6 on T lymphocytes, B lymphocytes, and monocytes, and ALCAM antibodies decreased the movement of monocytes, B cells, and $CD4^+$ T cells, but not $CD8^+$ T cells across BBB monolayer in vitro, and decreased the severity of EAE in vivo (Cayrol et al., 2008). In addition, monocyte infiltration into the CNS is important for the pathogenesis of MS. Studies have identified that migrating monocytes are induced by cytokines from the inflamed BBB to form antigen-presenting DCs, which can activate T cells (Bailey et al., 2007; Greter et al., 2005; Ifergan et al., 2008). Monocyte-endothelial cell coculture experiments have elucidated several steps in the mechanism by which monocytes can passage through the BBB. Monocytes can induce tissue plasminogen activator (tPA) release by CNS endothelial cells, which activates ERK1 and ERK2 kinases and which can lead to a degradation of the tight junction protein Occludin (Reijerkerk et al., 2006, 2008).

These findings demonstrate that neuroinflammatory processes can lead to a breakdown of the BBB and an increased leukocyte migration into the CNS. This leukocyte infiltration causes massive damage to the CNS, and thus, methods to inhibit excess inflammation may prove vital for therapeutics for these diseases. However, the death of Natalizumab-treated patients because of the viral Progressive Multifocal Leukoencephalopathy (PML) highlights the importance of a basal level of immune surveillance in clearing CNS pathogens (Engelhardt, 2008). Therefore, the BBB performs a crucial role in regulating the degree of CNS immune surveillance.

Evasion of Barriers by Pathogens

Although the two barriers provide an effective obstacle, specific pathogens, including bacteria, fungi, and viruses, have developed mechanisms that utilize cellular and molecular components of the host immune system and/or the barriers themselves to gain access to the CNS. Methods of crossing the barriers include transcellular passage through tight junctions, paracellular passage in endosomal vesicles, and "Trojan horse" approaches by which invasive microorganisms infect bone marrow-derived cells that can cross the barriers (Kim, 2008). In addition pathogens themselves can alter the function of the barrier and cross "leaky" barriers (Figure 2).

Although the major portal of entry for invasive pathogens across the gut mucosa are the M cells that are interspersed in the follicle-associated epithelium of Peyer's Patches and

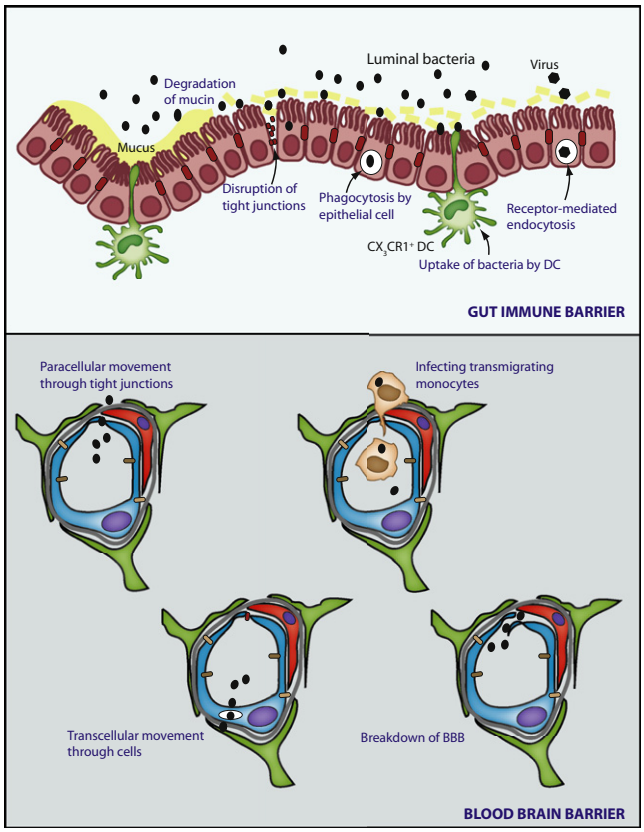

Figure 2. Mechanisms Developed by Bacteria to Cross the GIB or the BBB

Bacteria have evolved strategies to degrade mucins and to cross the epithelial barrier. Invasive bacteria can release proteases or glycopeptidases to degrade mucins, which then allows them to penetrate the epithelial barrier. Four alternative pathways have been described: Bacteria can target TJ proteins either directly or via the release of toxins to disorganize TJ allowing their penetration via the paracellular route. Bacteria can induce their own phagocytosis by epithelial cells. Microorganisms can also be taken up directly by intraepithelial DCs. Microorganisms such as viruses can exploit the receptor-mediated endocytosis. In the CNS, pathogens, including viruses and bacteria, can evade the BBB by paracellular movement through tight junctions, or transcellular movement through the cells (often in membrane bound vacuoles), or through a Trojan horse approach by infecting transmigrating monocytes, or by eliciting a breakdown of the BBB, thus allowing free entry into the CNS.

isolated lymphoid follicles, virulent microorganisms have developed several strategies to invade the GIB. First, they can degrade the mucous layer. Several microorganisms are equipped with proteases and glycosidases able to digest the mucins (Crowther et al., 1987; de Repentigny et al., 2000; Moncada et al., 2000). Second, they have the capacity to penetrate the epithelial barrier. This can be achieved via four alternative pathways (Figure 2). Bacteria such as *Salmonella*, *Shigella*, and *Yersinia* can induce their own phagocytosis by epithelial cells (Cossart and Sansonetti, 2004)—a characteristic that is conferred by type-three secretion systems (a syringe-like apparatus that allows the injection of virulence factors that induce cytoskeleton rearrangements and bacterial engulfment) (Mueller et al., 2008); Microorganisms can also be taken up directly by intraepithelial DCs that are naturally phagocytic cells (Niess et al., 2005; Rescigno et al., 2001) but that also have the ability to translocate to mesenteric lymph nodes for antigen presentation and T cell activation; microorganisms such as *C. difficile*, *B. fragilis*, *V.cholera*, and *C. perfringens* can target TJ proteins either directly or via the release of elaborated toxins and disorganize TJ allowing their penetration via the paracellular route (Berkes et al., 2003); and finally, viruses can exploit receptor-mediated endocytosis in epithelial cells and likely also DCs (Fleeton et al., 2004; Gastaldelli et al., 2008; Turville et al., 2002). Once inside the host, bacteria are attacked by first-line resident and recruited immune cells that either release antimicrobial mediators or phagocytose the microorganisms for their killing. However, several bacteria have evolved strategies to evade phagocytic killing and to survive within phagocytes. Phagocytes are motile cells and can be used as "Trojan horses" to invade other organs such as the spleen (Vazquez-Torres et al., 1999), liver, and eventually, the brain (Buckner et al., 2006). It remains to be established whether the lamina propria capillaries that are located just underneath the basal membrane of epithelial cells have the capacity to impede the entrance of microbes into the systemic circulation and whether this barrier is evaded by invasive bacteria.

Infections are quite common, but why do we only see infections of the CNS in rare occasions? One factor is the magnitude of bacteraemia. Similar blood amounts of *Eschericia coli* were required for passage across the BBB in animals of different ages suggesting a threshold level of bacteraemia must be reached before crossing BBB (Kim et al., 1992). This has also been shown for other pathogens including *Streptococcus agalactiae* and *Streptococcus pneumonia* (Kim, 2008) and highlights the importance of external barriers in limiting the serum amounts of pathogens.

Studies have utilized cultured human brain microvascular endothelial cells (HBMECs) to identify the mechanisms of BBB transversal. Several pathogens, including *Trypanosoma* and *Borrelia*, have been shown to cross the HBMECs through a paracellular route (Kim, 2008). Electron microscopy (EM) studies demonstrate that *Trypanosoma brucei gambiense* and *Borrelia burgdorferi* bind to endothelial cells at or near the tight junctions (Grab et al., 2004, 2005), and passage of *Trypanosoma brucei gambiense* through the endothelial cell layer causes a decrease in transendothelial cell electrical resistance (Grab et al., 2004). Furthemore, attachment of *Borrelia* causes an increase in vascular matrix metalloproteinases expression (Grab et al., 2005), which has been shown to degrade tight junctions allowing for passage of monocytes (Reijerkerk et al., 2006). *Neisseria meningitides* also cross endothelial cells through parecellular junctions. These bacteria possess type IV pili, which bind human brain endothelial cells and recruit the Par3-Par6-PKCζ polarity complexes away from tight junctions to bacterial-cell interfaces. This process leads to disruption of paracellular junctions and allows access of the pathogen to the CNS (Coureuil et al., 2009).

In addition, pathogens including *E. Coli*, *S agalactiae*, *S. pneumonia*, *Neisseria meningititis*, *Candida albicans*, and *C. neoformans* can cross the BBB through a transcellular route (Kim, 2008). EM studies have visualized *E. coli* in membrane-bound vacuoles within HBMECs, suggesting a transcellular route of BBB crossing (Prasadarao et al., 1999). This passage involves binding to endothelial cell surface, alterations in endothelial

cytoskeleton forming membrane protrusions which encircle the bacteria, and traversal through endothelial cells in vacuoles while avoiding lysosomal fusion. Interestingly, K1 *E. coli* have been shown to adhere to HBMECS, but not endothelial cells derived from non-neural tissues, suggesting that there may specific targeting of this strain to neural tissue (Kim, 2008). Mutagensis screening has identified several *E. coli* genes that affect its passage across the BBB into the CNS. Specifically the K1 variant of *E. coli* causes meningitis and strains deleted for *ompa*, *fimh*, *ibea*, *ibeb*, *ibet*, *yijp*, *asla*, and *cnf1* are less likely to cross BBB (Hoffman et al., 2000; Huang et al., 1995, 1999; Khan et al., 2007; Teng et al., 2005, 2006; Wang et al., 1999; Zou et al., 2007). Several details of the molecular mechanism for *E. coli* binding to HBMECs have been elucidated. For instance, the outer membrane protein OmpA binds to GlcNAc residues on endothelial surface glycoproteins including gp96, and also increase the expression of type 1 fimbriae, which bind CD48 on endothelial cells (Khan et al., 2007; Prasadarao et al., 2003; Teng et al., 2006). Invasion of endothelial cells requires IbeA proteins that bind PSF and activate cytoskeletal changes in a process that ''zipper'' the bacteria (Zou et al., 2007). CNF1 is a bacterial $\alpha\beta$ toxin that aids in the traversal through endothelial cells by activation of RhoGTPases (Chung et al., 2003). Interestingly, CNF1 acts through the laminin receptor on endothelial cells, which is also a cellular target for dengue virus, adeno-associated virus, prion protein, *S pneumonaie*, *N. meningitides*, and *Haemophilus influenzae* (Kim, 2008; Orihuela et al., 2009). This suggests that evolutionary diverse pathogens have developed similar methods to cross the BBB.

HIV may be the best example of a pathogen that crosses the BBB by a Trojan horse mechanism because the virus infects monocytes that repopulate perivascular macrophages (Buckner et al., 2006). HIV has been shown to infect monocytes by interactions between viral envelope glycoproteins and monocyte CCR5 receptors (Deng et al., 1996). In addition, HIV infection of monocyte cells increases surface expression of LFA-1, an ICAM1 ligand that increases likelihood of adhesion to CNS endothelial cells (Stent and Crowe, 1997). Once in the CNS, monocytes can spread the virus to microglia, perivascular macrophages, and astrocytes, creating a reservoir of the virus. Patients with HIV have serious CNS complications, including encephalitis and dementia, which are characterized by leukocyte infiltration into the CNS, microglial activation, BBB disruption, and ultimately damage to neurons (Buckner et al., 2006). These symptoms are due to direct infection of the CNS, as parenchymal viral reservoirs are observed in patients. In fact, the prevalence of neurological symptoms in HIV patients is increasing because as new therapeutics extend lifespan by reducing viral loads in non-CNS tissue, these same therapeutics fail to cross the BBB and treat HIV reservoirs in the CNS.

In addition, pathogens can gain access to the CNS by disrupting the BBB. For instance, the HIV envelope glycoprotein gp120 has been shown to increase BBB permeability through interactions with endothelial transmembrane coreceptors CCR5 and CXCR4 (Kanmogne et al., 2007).

Concluding Comments

Although the GIB and BBB protect different tissues, these barriers have developed many similar mechanisms to maintain tissue homeostasis. Both barriers consist of a cellular layer that forms a physical barrier and a different array of immune cells that provide further protection. In each case, these barriers are regulated by interactions with neighboring cells and environmental stimuli, and are disrupted during pathological conditions. Their unique properties allow each barrier to deal with specific microenvironments and control the milieu of each tissue. Due to the remarkable similarities of barrier properties, studies on one barrier may provide important insight on the other barrier. For example, study of Occludin-Claudin-based tight junction strands in epithelial cells has led to understanding of tight junction strands in endothelial cells. Comparison of the barriers may also be very important in understanding disease because each barrier undergoes similar changes during pathological inflammation. For instance, patients with IBD have a higher incidence of MS, suggesting that these two autoimmune diseases that affect completely different tissues actually share similar mechanisms (Kimura et al., 2000) or common effectors. Perhaps a critical step in the onset of each disease is breakdown of the barrier mechanism that affords immune privilege to the tissue.

An important point is that neither barrier functions alone, but within the context of an organism, and thus, the function of each barrier may influence the other. For instance, food molecules that pass through the GIB into the blood stream can act on the BBB, and CNS molecules that leave the brain can access the GIB. Thus, it is important not only to study each barrier in isolation, but in the context of the organisms in order to understand how barriers including the BBB, GIB, skin, lung, kidney, and others work together to maintain homeostasis. Interestingly, both the GIB and BBB are regulated by interactions between barrier cells with glial cells that are connected with the enteric nervous system and the CNS, respectively. This neural coupling may provide a unique mechanism for these barriers to talk to each other. For instance, stress is known to influence GIB permeability (Gareau et al., 2008) and to exacerbate symptoms of IBS and IBD. Stress could affect mucosal barrier integrity via the action of the enteric nervous system on mast cells, glial cells, or enterochromaffin cells. While the effects on glial and enterochromaffin cells may remain local, mast cell degranulation and activation could lead to a systemic inflammatory response that could affect the permeability of the BBB. As mentioned above, mast cells are located on the brain side of the BBB and are ensheathed by astrocytes but, if activated, can also penetrate the BBB of adult rats (Silverman et al., 2000). Hence, under stress conditions, mast cells may migrate into the brain and participate to altered BBB permeability. What could be the consequences of an altered GIB and BBB? An interesting hypothesis has been recently proposed (Theoharides et al., 2008). A correlation between children with autistic spectrum disorders and anxiety, allergy, or food intolerance has been observed. Becuase mast cells can mediate allergic disorders and respond to stress, it was suggested that mast cells may mediate neuroinflammation and increased BBB permeability.

Thus, understanding the mechanisms that control the formation and function of each barrier, their interrelationship, and how pathogens evade them, may provide new insight into complex behavioral diseases and new targets for therapeutics to control infectious diseases.

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