Concepts and Mechanisms: Crossing Host Barriers

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The human body is bordered by the skin and mucosa, which are the cellular barriers that define the frontier between the internal milieu and the external nonsterile environment. Additional cellular barriers, such as the placental and the blood–brain barriers, define protected niches within the host. In addition to their physiological roles, these host barriers provide both physical and immune defense against microbial infection. Yet, many pathogens have evolved elaborated mechanisms to target this line of defense, resulting in a microbial invasion of cells constitutive of host barriers, disruption of barrier integrity, and systemic dissemination and invasion of deeper tissues. Here we review representative examples of microbial interactions with human barriers, including the intestinal, placental, and blood–brain barriers, and discuss how these microbes adhere to, invade, breach, or compromise these barriers.

INVASIVE PATHOGENS AND HOST BARRIER

A critical event in systemic infection is the crossing by its causative agent of one or multiple host barriers (Fig. 1). It can schematically occur according to two nonexclusive scenarios: (i) the barrier function of a tissue can be compromised either by physical means (wound, catheter, surgery, arthropod bite) or in the context of a host condition that disrupts the integrity of tissues (inflammation, dysfunction of a host gene product implicated in bar-

rier function), and this barrier damage allows microbial invasion; (ii) barrier(s) can also be crossed actively, via the direct action of microbial gene products that mediate microbial adhesion to and translocation across cells constitutive of host barriers.

ENTEROPATHOGENS AND THE INTESTINAL BARRIER

The skin and mucosa form the barriers that separate the host from its external nonsterile

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Figure 1. Example of host barriers. From *top to bottom*: The blood–brain barrier is made by brain endothelial cells that express tight junctions. The blood–CSF barrier is constituted by a monolayer of epithelial cells from the choroid plexus, which separates the blood located in the choroid plexus from the cerebrospinal fluid. The intestinal barrier is a mucosal barrier made by a monolayer of epithelial cells. The placental barrier is constituted by both syncytial trophoblastic cells and mononuclear cytotrophoblasts.

environment. The intestinal barrier is made of a monolayer of polarized epithelial cells called enterocytes. Tight junctions (TJs) and adherens junctions (AJs) hold together enterocytes. TJs are the most apical structures and are made of transmembrane proteins (occludins, claudins, junction adhesion molecules [JAMs], and the coxsackievirus and adenovirus receptor [CAR]), connected to intracellular proteins (such as ZO-1, -2, -3, MAGUK, and PAR family proteins). TJs prevent paracellular diffusion of molecules or luminal microorganisms (Anderson et al. 2004). AJs are required for the integrity of TJs and are located directly below them (Harris and Tepass 2010). They are made of E-cadherin, which is connected to the actin cytoskeleton. AJs and TJs are located at the apical and luminal side of enterocytes. Their basolateral side seats on a basement membrane made of extracellular

matrix proteins, which separate them from the lamina propria, that contains connective tissue, stromal cells, blood and lymphatic capillaries, and cellular effectors of the immune system, such as lymphocytes, dendritic cells, or resident macrophages, some of which are in direct contact with enterocytes and the intestinal lumen via extending dendrites (Rescigno and Di Sabatino 2009).

The intestinal epithelium apical surface delineates the interface between the host and the intestinal lumen. The intestinal barrier is in continuous contact and crosstalk with up to 10^{14} microorganisms, which constitute the intestinal microbiota. It represents a major potential portal entry for microbes into the host. Microbial pathogens have evolved specific capacities to adhere to, invade, or disrupt the intestinal barrier (Sousa et al. 2005; Guttman and Finlay

2009; Yoshida et al. 2011). These pathogens have to counteract host defenses and competition from the resident microflora in the gut. Microbes can cross epithelial barriers by disrupting intercellular junctions or by interacting with epithelial cell receptors, leading to the traversal in a transcellular or paracellular route. Pathogens may also take advantage of the luminal sampling property of host cells to get access to the lamina propria.

Disruption of Tight Junctions by Enteropathogens

Intestinal pathogens may adhere to the apical surface of enterocytes, remain extracellular, and modify the barrier properties of the epithelium. This event can lead to local invasion of the lamina propria. Enteropathogenic and enterohemorrhagic *E. coli* (EPEC and EHEC) are diarrheagenic bacteria (Wong et al. 2011) that affect TJs (occluding and ZO-1) through type-III secretion system (TTSS) effectors EspF, Map, and EspG (Dean and Kenny 2004; Matsuzawa et al. 2005), leading to a decreased transepithelial resistance and aberrant TJs. *Helicobacter pylori* expresses several adhesins that mediate bacterial attachment to gastric epithelial glycan receptors, and possesses a type-IV secretion system that injects the multifunctional bacterial effector CagA into the host cell cytoplasm (Rieder et al. 2005). CagA alters the composition and the function of TJs (Amieva et al. 2003) and leads to a disruption of the epithelial polarity, which could have a role in *H. pylori*-associated carcinogenesis (Saadat et al. 2007).

Toxin A and toxin B from *Clostridium difficile* are both implicated in the pathogenesis of pseudomembranous colitis (Kuehne et al. 2010). They dissociate TJ's proteins occludin, ZO-1 and ZO-2, thus increasing intestinal epithelium permeability (Voth and Ballard 2005). The bifunctional enterotoxin CPE of *Clostridium perfringens*, which can cause diarrhea, forms pores in the host cell plasma membrane, and binds claudin-3 and claudin-4 with high affinity (Sonoda et al. 1999; Winkler et al. 2009; Robertson et al. 2010), inducing their degradation, a decrease in transepithelial resistance

and disruption of TJs. The role of claudins in the barrier function of TJs was discovered via these studies (Sonoda et al. 1999).

Rotavirus, member of the Reovirus family causing gastroenteritis, also colonizes the gut and disrupts tight junctions. VP8 subunit of VP4, an outer rotavirus protein, moves ZO-1, claudin-3, and occludin from TJs, then disrupting the barrier integrity (Nava et al. 2004; Beau et al. 2007). Similar effects are produced by the viral enterotoxin NSP4, which blocks the formation of TJs by preventing lateral recruitment of ZO-1 (Tafazoli et al. 2001). Reoviruses use the TJ protein JAM-1 as a cellular receptor (Barton et al. 2001). The viral surface protein $\sigma 1$ directly interacts with the amino-terminal domain of JAM-1 and disrupts the homodimers of this junction protein (Forrest et al. 2003; Campbell et al. 2005; Guglielmi et al. 2007).

Active Microbial Crossing of the Intestinal Barrier

Translocation across Enterocytes

Listeria monocytogenes is a facultative intracellular bacterium that crosses the intestinal, blood–brain and placental barriers, inducing gastroenteritis, meningitis/encephalitis, and materno-fetal infection. InlA and InlB, two surface proteins of *L. monocytogenes*, which interact with their respective host receptors E-cadherin and Met, induce internalization of *L. monocytogenes* into cultured epithelial cells. Importantly, in contrast to human E-cadherin, mouse E-cadherin is unable to interact with InlA and to promote *L. monocytogenes* entry into cells (Lecuit et al. 1999, 2001). This species specificity relies on the sixteenth amino acid of the mature E-cadherin, a proline in humans, and a glutamic acid in mice. E-cadherin from guinea pig is also recognized by InlA and harbors a proline at position 16. In this species, contrary to the mouse, *L. monocytogenes* is able to cross the intestinal barrier, disseminate systemically, and induce a dose- and InlA-dependent lethality (Lecuit et al. 2001). To prove the role of the InlA–E-cadherin interaction in the ability of *L. monocytogenes* to cross the intestinal barrier, a transgenic mouse model expressing human E-cadherin in post-

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mitotic nonproliferative small intestinal enterocytes was designed (Lecuit et al. 2001). In contrast to nontransgenic mice, and similarly to guinea pigs and humans, transgenic mice expressing human E-cadherin are highly permissive to orally acquired listeriosis, demonstrating a critical role for InlA in the ability of *L. monocytogenes* to cross the intestinal barrier. Epidemiological study of human cases of listeriosis evidence supports this result, because clinical strains express a functional InlA significantly more often (96%) than food isolates do (65%) (Jacquet et al. 2004), which is in favor of a role of InlA in crossing the intestinal barrier in humans. In contrast to the InlA–E-cadherin interaction, the InlB–Met interaction is not necessary for crossing the intestinal barrier in transgenic mice, as well as in gerbil, which is permissive for InlA and InlB (Khelef et al. 2006; Disson et al. 2008). The way by which *L. monocytogenes* gets access to the adherens junction protein E-cadherin and the mechanism by which bacteria cross the intestinal barrier were not known until recently. It had been proposed that *L. monocytogenes* access to E-cadherin may be facilitated when extruding epithelial cells detach from the tips of intestinal microvilli, thereby exposing E-cadherin to the luminal side of the intestine (Pentecost et al. 2006). This was shown in vitro and correlated with a colocalization of bacteria with extruding cells in rabbit ileal loop in vivo. It has now been shown that E-cadherin is lumenally accessible not only around extruding enterocytes at the tip and lateral sides of villi, but also particularly around mucus-expelling goblet cells (GCs) and in villus epithelial folds. *L. monocytogenes* preferentially adheres to accessible E-cadherin on GCs in a ligated loop of transgenic mice expressing human E-cadherin, is internalized, rapidly transcytosed across the intestinal epithelium, and released in the lamina propria by exocytosis from where it disseminates systemically (Nikitas et al. 2011). *L. monocytogenes* is thus able to exploit intrinsic tissue heterogeneity to access its receptor. These results highlight transcytosis as an unexpected pathway that is hijacked by *L. monocytogenes* to breach the intestinal barrier and cause systemic infection. The precise mechanism by which *L. mono-*

cytogenes transcytoses across enterocytes is currently being investigated.

Some viral species also use basolateral cell adhesion molecules as receptors to infect epithelia. The coxsackievirus and adenovirus receptor (CAR) is a component of the tight junction, which is located under the classical TJ proteins (occludin and claudin) and is thus inaccessible to viruses that contact host cells via their apical surface (Freimuth et al. 2008). As indicated by its name, it is both targeted by adenovirus and coxsackieviruses, leading to a decrease in epithelial impermeability (Bergelson et al. 1997). Coxsackieviruses B are members of the Enterovirus family and the causative agents of meningitis and myocarditis. Coxsackievirus-B infects polarized cell layers through binding to the decay-accelerating factor (DAF) proteins on the apical cell surface (Coyne and Bergelson 2006). Virus attachment to DAF leads to the activation of the src kinase Abl, inducing a Rac-dependent reorganization of the actin cytoskeleton that enables the viruses to relocate to tight junctions. Once in the junction, viral particles interact with CAR, and are internalized in caveolar vesicles, a mechanism dependent on the activation of Fyn kinase by DAF (Coyne and Bergelson 2006). Internalization of coxsackievirus-B also requires the TJ protein occludin (Coyne et al. 2007). The subsequent release from epithelial cells has been suggested to occur through a calcium signal and calpain-dependent necrosis of the infected cells (Bozym et al. 2011).

Host Cell Sensing of Intestinal Lumen Content as an Achilles' Heel Exploited by Microbes

M cells, which are specialized epithelial cells located at the Peyer's patch level and in villi and CD11c⁺CX3CR1^{high} cells, continuously sample the intestinal lumen and transport antigens to the mucosal lymphoid tissues (Rescigno 2011). This process is required to either induce tolerance or to initiate immune responses. However, many microbes exploit M cells as an Achilles' heel to enter the lamina propria, including enteroviruses, *Yersinia enterocolitica*, *Shigella flexneri*, and *Salmonella enterica* (Corr et al. 2008).

S. enterica causes severe diseases in humans, ranging from gastroenteritis to systemic infections (typhoid fever). Systemic infections because of *Salmonella* serovars are species-specific: *S. enterica* serovar Typhi (*S. Typhi*) induce typhoid fever, a systemic infection in human, whereas *S. enterica* serovar Typhimurium (*S. Typhimurium*), which induces a self-limited gastroenteritis in immunocompetent humans, but causes a typhoid-like systemic infection in mice. For this reason, mice have been widely used to study typhoid fever (Monack et al. 2004), although they are generally more resistant than humans to intestinal colonization by *S. enterica*. To circumvent this limitation, a streptomycin pretreated mouse model of enterocolitis has been developed that allows probing the host and bacterial factors contributing to intestinal immunopathology (Grassl and Finlay 2008). After oral ingestion, *S. Typhimurium* invades intestinal tissues and induces mucosal inflammation. It may enter the blood circulation and infect downstream organs such as liver and spleen (Watson and Holden 2010). *S. Typhimurium* exploits both dendritic cells and M cells to access the lamina propria. It was shown that, in the terminal ileum of mice, CD11c⁺ (mainly CX3CR1^{high}) lamina propria monocytic phagocytes extend protrusions across the epithelium, which facilitates pathogen transport from the gut lumen into the lamina propria (Rescigno et al. 2001; Niess et al. 2005; Chieppa et al. 2006). DCs may then allow the clearance of the pathogen, but in an immunocompromised host may lead to the subsequent colonization of deeper organs. However, sampling by DCs alone does not account for the translocation of *S. Typhimurium* to the lamina propria (Hapfelmeier et al. 2008). *S. Typhimurium* is also able to translocate through M cells to access Peyer's patches lamina propria (Jones et al. 1994). The glycoprotein 2 (GP2), specifically expressed on the apical plasma membrane of M cells, recognizes FimH, a component of type-I pili expressed by *Enterobacteriaceae*, including *Escherichia coli* and *S. Typhimurium*, which allows the internalization of these bacteria (Hase et al. 2009). Another protein, encoded by *lpf* from the fimbrial operon, interacts with mouse M

cells (Baumler et al. 1996). Once attached, *Salmonella* pathogenicity island 1 (SPI-1), which codes for bacterial proteins translocated into target cells by the SPI-1 type-III secretion system (TTSS-1), promotes bacterial invasion of mouse M cells (Clark et al. 1998). *S. Typhimurium* may also cross the intestinal barrier through the epithelial cells in an SPI-1 dependent manner. Recently, the mechanism by which *S. Typhimurium* crosses the epithelial barrier at the cecal level and is released in the lamina propria has been studied in detail (Muller et al. 2012). It has been shown by use of in vivo confocal microscopy in a murine *Salmonella*–diarrhea model that the breaching of the epithelial barrier relies on both TTSS-1-mediated invasion and TTSS-2-dependent transcytosis to the basolateral side. Once in the lamina propria, the bacterium is transiently extracellular before being phagocytized, among others by CD11c⁺CX3CR1^{high} monocytic phagocytes, which enables it to colonize the lamina propria. *S. Typhimurium* may then exploit the immune surveillance after crossing the epithelial barrier to infect its host.

Paracellular Pathway

Pathogens such as parasites (*Entamoeba histolytica* and *Toxoplasma gondii*) (Yoshida et al. 2011) or bacteria (*Streptococcus agalactiae*) have been proposed to cross the intestinal barrier in a paracellular route. This serves to target intercellular junctions and to actively propel between two cell membranes. *Toxoplasma gondii* (*Tg*) is a human parasite with the capacity to actively cross not only the intestinal barrier but also the blood–brain and placental barriers (Barragan and Sibley 2003). *Tg* parasites have been shown to accumulate around intercellular junctions. They may use the paracellular route to cross epithelial barriers without altering the barrier permeability. Crossing may occur through the interaction of the *Tg* surface adhesin MIC2 with the intercellular adhesion molecule 1 (ICAM-1), which is present in the apical surfaces of epithelial barriers (Barragan et al. 2005) and requires the motility property of the parasite (Barragan and Sibley 2002). *Tg* has to be inter-

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nalized by host cells to replicate, and its entry and the formation of a parasitophorous vacuole depend on the association of its apical tip with the host cell through the parasite transmembrane protein AMA1, and a subsequent rearrangement of the host and Tg cytoskeletons. This allows the parasite to replicate at the intestinal level, and could also be the first step for the invasion of downstream organs (Tyler and Boothroyd 2011).

Streptococcus agalactiae (group B *Streptococcus* [GBS]) is a normal constituent of the intestinal microflora and the major cause of human neonatal meningitis. Among the GBS clones, ST-17 is strongly associated with meningitis in infants. It has been shown that a ST-17-specific surface-anchored protein called hypervirulent GBS adhesin (HvgA) is required for intestinal colonization and translocation across the intestinal barrier and the blood–brain barrier (BBB), leading to meningitis (Tazi et al. 2010). The mechanism by which the ST-17 clone crosses the intestinal barrier is still unknown. However, previous data suggest that the paracellular route could be a way for GBS to cross epithelial barriers. Using differentiated epithelial cells grown on Transwell inserts as a model, it has been shown by transmission electron microscopy that GBS interacts with intercellular junctions and has the capacity to cross the monolayer by a paracellular mechanism via an active and transient opening of cell junctions (Soriani et al. 2006). This process depends on the pilus backbone of GBS, but the host receptors are still not known (Pezzicoli et al. 2008). However, the mechanism by which the ST-17 clone crosses the intestinal barrier is currently unknown.

MICROBES AND THE PLACENTAL BARRIER

The human maternofetal barrier contains two anatomically distinct components, the chorioallantoic placenta and the chorioamnion. The barrier is formed at the placental level by specialized epithelial cells, the villous syncytiotrophoblasts, which are differentiated from underlying mononuclear cytotrophoblasts. Multinucleated syncytiotrophoblasts are in direct contact with maternal blood circulating in the intervillous

space. A basement membrane separates these trophoblastic cells from a connective tissue that contains fetal capillaries. The amniotic epithelium forms the maternofetal interface in the chorioamnion. The apical surface of this epithelium is exposed to amniotic fluid, whereas its basal surface sits on a basement membrane that overlies the amniotic mesoderm (Cross et al. 1994; Huppertz 2008; Maltepe et al. 2010).

A number of pathogens invade the placenta but few are known to actively cross the placental barrier and disseminate to the fetus. Infection of placenta can lead to abortion, premature delivery, and fetal and neonatal infection. *Coxiella burnettii*, the agent of Q fever, is an intracellular bacterium which may infect trophoblast (Baumgartner and Bachmann 1992; Ben Amara et al. 2010) and, in rare cases, the fetus (Carcopino et al. 2009) leading to abortion and premature delivery. *Plasmodium falciparum*, the etiological agent of the malaria, has long been known to affect pregnancy (Brabin et al. 2004). Parasitized erythrocytes bind to chondroitin sulfate A expressed at the syncytiotrophoblast apical surface, where they accumulate, impeding the physiological functions of the placenta (Scherf et al. 2001). Adverse outcomes are low birth weight babies, fetal loss, as well as increased perinatal and maternal mortality. Several Chlamydia spp, such as *Chlamydia trachomatis*, *Chlamydo-phil a abortus*, *Chlamydo-phil a psittaci*, and *Chlamydo-phil a pneumonia* are involved in adverse pregnancy outcomes in humans and/or animals (Baud et al. 2008). These obligate intracellular bacteria have been shown to infect trophoblasts in a mouse model of infection, probably via infected maternal neutrophils (Buendia et al. 1998).

The exact mechanism by which pathogens may cross the placental barrier and infect the fetus remain often elusive. The mechanisms by which some of the microorganisms responsible for maternofetal infections in human may cross this barrier are described below.

Tg is responsible for congenital infection in up to 30% of infected pregnant women, when primary infection is acquired during pregnancy. Tg has been shown to infect directly primary human trophoblasts (Abbasi et al. 2003) and

extravillous trophoblasts of human ex vivo inoculated placental explants (Robbins et al. 2012), as well as trophoblasts in a rodent animal model (*Calomys callosus*) (Ferro et al. 2002). This invasion may be the critical event leading to the infection of the fetus. Another nonexclusive hypothesis is that *Tg* crosses the placental barrier in a paracellular way through the interaction between the parasite adhesin MIC2 and ICAM-1 (Barragan et al. 2005), ICAM-1 being up-regulated during the infection (Juliano et al. 2006).

Brucella abortus (*Ba*) is an intracellular bacterium that infects animals and humans, inducing a fetal abortion. *Ba* is thought to be transmitted in cows via the oro-pharyngeal route after exposure to infected placenta released on abortion. It colonizes placenta in its natural hosts (Carvalho Neta et al. 2010) as well as giant trophoblasts in a mouse animal model (Tobias et al. 1993; Kim et al. 2005b). It could be transmitted to the fetus, indicating a crossing of the placental barrier. A surface protein of *Ba* (SP41) is associated with adherence to and invasion of nonphagocytic HeLa cells (Castaneda-Roldan et al. 2006). However, the receptor is not known and trophoblasts may express other receptors recognized by *Ba*. The exact mechanism by which *Ba* may cross the placental barrier is not known.

Coxsackieviruses B (CVB) may be transmitted to the fetus during delivery (Moore 1982) but the rate of transplacental transmission although present (Konstantinidou et al. 2007), is not known. In a study conducted by Satosar et al. (2004) it was shown that CVB was present in 35% of infected placentae obtained from cases with fetal or neonatal death or severe neonatal morbidity, indicating a potential strong role of coxsackieviruses in placental infection. It is not known, however, if the mechanism by which CVB crosses the fetal barrier has similarities with crossing of other host barriers (intestinal barrier and BBB).

Rubella virus, which belongs to the *Togaviridae* family, is associated with congenital rubella syndrome (CRS), a major cause of developmental abnormalities, including blindness and deafness (Banatvala and Brown 2004; Duszak 2009).

Despite efficient vaccination programs, this disease still affects some areas in developing countries. Infection by Rubella virus occurs by inhalation, the placenta being subsequently infected, leading to focal necrosis of the epithelium in the chorionic villi and in the endothelial cells of its capillaries (Tondury and Smith 1966). Despite the existence of animal models of vertical transmission (Cotlier et al. 1968; Kono et al. 1969), the pathophysiology of the infection remains mostly elusive. It has been proposed that infected cells in the placenta detach into the lumen of vessels, resulting in infection and damage of fetal organs. As classically observed for vertically transmitted microbes, symptoms associated with CRS are more severe when the infection occurs during the first trimester of gestation but the infection can occur throughout the pregnancy (Grillner et al. 1983; Miller 1991).

The recent reemergence of another member of the *Togaviridae* family that belongs to the *Alphavirus* genus, Chikungunya virus, led to the demonstration that it is associated with severe neonatal infections, including encephalopathy. As mother-to-child transmission is rare in antepartum but frequent in the context of intrapartum maternal viremia, and the placenta is not a site of viral replication, this favors a model of vertical transmission via placental breaches that forms around the term during parturition, as described for a number of other viruses associated with elevated maternal viremia during delivery, such as HIV, HBV, and arboviruses such as Dengue virus or West Nile virus (Couderc et al. 2008; Gerardin et al. 2008).

L. monocytogenes actively crosses the placental barrier and causes severe maternofetal infections in humans (Mylonakis et al. 2002; Lecuit et al. 2007). The ability of *L. monocytogenes* to infect cytotrophoblastic and syncytiotrophoblastic cells has been shown from immunohistopathological studies of placentas from women with listeriosis, in conjunction with the use of human primary trophoblasts and third trimester human placental explants (Lecuit et al. 2004). Importantly, and in contrast to what is observed at the intestinal barrier level, both InlA and InlB

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interactions with their receptors on syncytiotrophoblasts are necessary for efficient invasion (Lecuit et al. 2004). These findings suggest that *L. monocytogenes* crossing of the human fetoplacental unit follows a transplacental route, after attachment to and invasion of the syncytiotrophoblasts in an InlA- and InlB-dependent manner, respectively. It has been recently proposed in an ex vivo model of human first trimester placental explant that *L. monocytogenes* enter the placenta in an InlA-dependent manner mostly through the extravillous trophoblasts, which anchor the placenta in the decidua, and not through the syncytiotrophoblasts (Robbins et al. 2010). This apparent difference may be because of intrinsic anatomical differences in first and third trimester explants, the former presenting with more anchoring villi and extravillous trophoblasts than the latter. Of note, infection of fetus and abortion because of *L. monocytogenes* occur far more frequently during later stages of pregnancy (~80% of maternofetal listeriosis occur after 22 weeks of amenorrhea; data from the French National Reference Center for *Listeria*). The requirement of InlA in *L. monocytogenes* crossing of the placental barriers has been independently shown epidemiologically, because *L. monocytogenes* isolates expressing a functional InlA are very significantly associated with their fetoplacental origin (Jacquet et al. 2004; Disson et al. 2008). The mechanism of infection has also been studied in animal models in vivo. As previously described, a limitation to the study of *L. monocytogenes* in vivo is that InlA and InlB interactions with their respective receptors are species-specific: InlA interacts with guinea pig E-cadherin, as in human, but not mouse and rat, E-cadherin (Lecuit et al. 1999), whereas InlB interacts with mouse Met, as in human but not guinea pig Met (Khelef et al. 2006). In apparent contradiction with the above epidemiological, ex vivo and in vitro results, InlA plays no role in *L. monocytogenes* fetoplacental infection in the guinea pig, despite its permissiveness to the InlA pathway (Bakardjiev et al. 2004). Similarly, InlB plays no role in *L. monocytogenes* fetoplacental infection in the mouse, despite its permissiveness to the InlB pathway (Le Monnier et al. 2007). To investigate

the role of both InlA and InlB in vivo, studies were conducted in two complementary animal models permissive for these two pathways: the gerbil, a natural host for *L. monocytogenes*, and a knockin mouse line that expresses a humanized version of E-cadherin in place of endogenous mouse E-cadherin. Using these two models, it has been established that *L. monocytogenes* specifically targets the placenta in vivo, in an InlA and InlB interdependent manner, and interacts with its receptors E-cadherin and Met, thereby deciphering the molecular mechanism underlying the ability of *L. monocytogenes* to target and cross the placental barrier (Disson et al. 2008).

CENTRAL NERVOUS SYSTEM PATHOGENS AND THE BLOOD–BRAIN BARRIER

Infectious meningitis is a severe and potentially lethal infection of the meninges, the protective membranes covering the central nervous system (CNS), even in otherwise healthy children or adults. Clinical and neuropathologic studies have clearly documented the complications of bacterial meningitis, which are brain edema, increased intracranial pressure, seizure, arterial and venous cerebral vascular insults, and other motor, sensitive, sensorial, and cognitive sequelae (Scheld et al. 2002). Host immune responses are frequently inadequately tuned to control infection within the CNS, either insufficient and unable to control microbial burden, or excessive and associated with excessive inflammation and irreversible tissue damages (Tunkel and Scheld 1995; Pfister and Scheld 1997). In this context, disruption of the BBB is a hallmark event in the pathophysiology of meningitis and subsequent CNS disease, which is critical not only for microbial access to the CNS, but also in the control of the infection.

The BBB maintains the homeostasis of the CNS microenvironment by restricting access of macromolecules, cells, and pathogens. The BBB *sensu stricto* is anatomically represented by the cerebral microvascular endothelium. Epithelial cells of the choroid plexus and meningeal vessels are two additional potential sites of crossing the blood–cerebrospinal fluid barrier. Brain micro-

vascular endothelial cells (BMEC) are held together by tight junctions, display a paucity of pinocytosis, and thus form a barrier (Betz 1985, 1992). Yet, despite its highly restrictive nature, certain pathogens are able to penetrate the BBB and gain entry into the CNS. The exact mechanisms by which these microorganisms pass through the BBB result from a complex interplay between host and microbial factors. Two principal mechanisms, operating individually or in tandem, are an increased permeability of the barrier and the direct invasion of the microorganism. The reasons for increased permeability of the BBB during infections are not fully understood. A dose- and time-dependent increase in permeability was observed in various animal models of bacterial meningitis (Temesvari et al. 1993; Kim et al. 1997; Mayhan 1998). These data suggest a role for bacterial factors, like cell wall components and toxins, in promoting BBB permeability either directly or indirectly through induction of host factors. Several reports have linked increased BBB permeability to a parallel increase in levels of circulating cytokines in the blood and CSF during infection (Lo et al. 1994). Tumor necrosis factor α (TNF- α), IL-1, and IL-6 are major early response cytokines that trigger a cascade of inflammatory mediators, including other cytokines, chemokines, arachidonic acid metabolites, and reactive nitrogen and oxygen intermediates (Dinarello 2000).

Blood–Brain Barrier Composition

The BBB, composed primarily of a specialized layer of BMEC, separates the brain and its surrounding tissues from the circulating blood, tightly regulating the flow of nutrients and molecules and thereby maintaining the proper biochemical conditions for normal brain function (Betz 1985, 1992). In addition, other periendothelial structures, such as pericytes, astrocytes, and a basement membrane, contribute to the BBB function (Abbott et al. 2010). These cells function in a coordinated fashion to regulate the passage of nutrients and other small lipophilic molecules while restricting the access of other macromolecules (Pfister et al. 1992; Rubin and

Staddon 1999). The brain endothelial cells are mainly characterized by the presence of tight intercellular junctions that promote high trans-endothelial electrical resistance and therefore impede paracellular flux of macromolecules (Ballabh et al. 2004). Similar to epithelial cells, endothelial cells express adherens junctions (AJ) and tight junctions (TJ) proteins at sites of intercellular contacts (Schulze and Firth 1993; Wolburg and Lippoldt 2002). In BMEC, TJ are composed of four integral membrane proteins: occludin, claudins, junctional adhesion molecules, and cell-selective adhesion molecules, which are linked through cytoplasmic proteins (zonula-occludin-1 (ZO-1), -2, -3, cingulin) to the actin cytoskeleton. AJ are composed of VE-cadherins, which are connected to actin cytoskeleton through catenins (Furuse 2010; Lampugnani et al. 1995; Hirase et al. 1997; Martin-Padura et al. 1998; Rudini and Dejana 2008). Claudin 1, -3, -5, and -12 have been identified at the BBB with claudin-5 being especially critical for BBB formation (Nitta et al. 2003), whereas occludin specifically regulates calcium flux between the blood and brain (Liebner et al. 2008). In addition to maintaining endothelial integrity for normal homeostasis these junctional complexes also play important roles in the maintenance of cell polarity, signaling, and modulation of transcription (Bazzoni and Dejana 2004; Dejana 2004).

Pathogen Invasion of the BBB

Significant progress has been made in understanding the molecular interactions between the BBB and microbial pathogens with the availability of in vitro tissue culture models of human BMEC (hBMEC) (Stins et al. 1994; Weksler et al. 2005) and in vivo animal models (Smith et al. 1973; Doran et al. 2002; Wickham et al. 2007; Uchiyama et al. 2009; Sheen et al. 2010). Microbial interactions with the BBB may involve crossing of BMEC in a vacuole (transcytosis), through the intercellular junctional spaces (paracytosis), or while inside a host cell (e.g., infected phagocyte) using it as a vehicle to cross the barrier (Trojan horse) (Kim 2008, 2010) (Fig. 2).

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Figure 2. (Top left) The blood–brain barrier (BBB) is composed of brain microvascular endothelial cells (BMEC), expressing tight junctions and surrounded by pericytes and astrocytes. From top to bottom and from left to right: Pathogens crossing of the BBB may occur in a vacuole (transcytosis), through intercellular junctional spaces (paracytosis), through an infected blood circulating cell (Trojan's Horse mechanism), or following the barrier disruption (BBB breakdown).

Microbial Adhesion to BMEC and Transcytosis across the BBB

Many meningeal pathogens are able to enter or “invade” brain endothelium apically and exit the cell on the basolateral side, thereby crossing the BBB cellularly. The engagement of host cell receptors results in signaling processes that drive massive host actin cytoskeletal rearrangement to promote bacterial uptake into the cell. Electron microscopic (EM) studies have shown the presences of meningeal pathogens in membrane bound vacuoles within BMEC, suggesting the involvement of endocytic pathways as well as avoidance of lysosomal fusion for BBB traversal (Nizet et al. 1997; van Sorge et al. 2008, 2011). Many studies have focused on the identifica-

tion of bacterial and host components involved in microbial interactions with the BBB (Kim 2008). Critical bacterial components have been discovered by screening random mutant libraries (Badger et al. 2000; Doran et al. 2005), analyzing bacterial transcription profiles during infection (Dietrich et al. 2003; Teng et al. 2005), and using bioinformatic approaches for whole genome comparisons (Uchiyama et al. 2009; van Sorge et al. 2009; Tazi et al. 2010). Bacterial pili, or fimbriae, have emerged as a common class of adhesins used by many meningeal pathogens, such as *E. coli* K1 (Teng et al. 2005), GBS (Maisey et al. 2007; van Sorge et al. 2009), and *N. meningitidis* (Kirchner and Meyer 2005) to initiate attachment to brain endothelium. Other well-characterized or recently described bac-

terial components such as CbpA and NanA, a surface anchored sialidase, in *S. pneumonia* (Ring et al. 1998; Uchiyama et al. 2009), lipoteichoic acid and HvgA in GBS (Doran et al. 2005; Tazi et al. 2010), type1 fimbriae, OmpA and ibeA/B in *E. coli* K1 (Prasadarao et al. 1999a,b; Teng et al. 2005), promote brain endothelial cell attachment and subsequent cellular penetration. Separately, bacterial toxins play an important role in microbial interactions with the BBB. In case of *E. coli* K1, cytotoxic necrotizing factor-1 (CNF-1) activates RhoA resulting in increased invasion of BMEC in vitro and increased penetration of the BBB in vivo (Khan et al. 2002, 2003). In contrast, β hemolysin (β -h/c) of GBS (Doran et al. 2003; Lembo et al. 2010), pneumolysin of *S. pneumonia* (Zysk et al. 2001) and HiB lipopolysaccharide (Patrick et al. 1992) all cause apoptosis in brain endothelial cells, which lead to increased BBB permeability and bacterial penetration in vivo.

A broad range of host cell surface receptors have been reported to mediate interaction with pathogens that lead to subsequent cellular penetration in the CNS. The laminin receptor (LR) and platelet activating factor receptor (PAFr) have been identified as common portals of CNS entry for the leading meningeal pathogens such as *E. coli* K1 (Chung et al. 2003; Kim et al. 2005a), *Neisseria meningitidis* (Virji et al. 1993), *Streptococcus pneumoniae* (Ring et al. 1998; Orihuela et al. 2009), and *Haemophilus influenzae* (Swords et al. 2001). LR was identified to function as a receptor for CNF-1 of *E. coli* K1 (Chung et al. 2003; Kim et al. 2005a), PilQ and PorA of *N. meningitidis*, CbpA of *S. pneumoniae*, and OmpP2 of *H. influenzae* (Orihuela et al. 2009). The PAFr binds to phosphorylcholine on the bacterial surface, which results in β -arrestin-mediated invasion of brain endothelial cells (Radin et al. 2005). Inflammatory activation of host cells shifts the targeting of *S. pneumoniae* to this G-protein-coupled receptor (Cundell et al. 1995). This results in promoting bacterial transcytosis across the cell, whereas non-PAFr-mediated entry shunts bacteria for exit and reentry on the apical surface in a novel recycling pathway (Ring et al. 1998). Integrins are also emerging as common receptors for

many meningeal pathogens. Bacteria may engage components of an extracellular matrix to bridge to integrins and initiate integrin signaling pathways. For example, human collagen bridges GBS pili adhesin, PilA, and $\alpha_2\beta_1$ integrin on BMEC, resulting in bacterial attachment, immune activation, and ultimately penetration of the CNS (Banerjee et al. 2011). Similarly, fibronectin bridges *N. meningitidis* to $\alpha_5\beta_1$ integrin on BMEC surface enabling bacterial internalization (Unkmeir et al. 2002). Glycan structures, specially glucosaminoglycans (GAG), that interact with integrin signaling machinery, have been shown to interact with bacterial proteins such as the GBS α C protein (Chang et al. 2011). Given that many of these host cell factors preferentially localize to the basolateral surface of polarized endothelium, disruption of junctional protein complexes on infection may result in a nonpolarized distribution of proteins on the BBB plasma membrane and their luminal accessibility (see below).

Paracytosis

Several meningeal pathogens are capable of either cleaving or remodeling these intercellular junctional proteins and therefore affect BBB integrity. Disruption of these cellular contacts leads to increased BBB permeability and also facilitates bacterial translocation into the brain. Bacterial toxins such as pneumolysin and β -hemolysin, of *S. pneumoniae* and GBS, respectively, are implicated in directly affecting the BBB integrity by causing endothelial cell death (Zysk et al. 2001; Lembo et al. 2010). For GBS, higher toxin production has been associated with increased capacity to cause meningitis (Doran et al. 2003). In addition, toxin production results in increased production of proinflammatory cytokines/chemokines, especially TNF- α , which negatively impact the BBB function (Sharief et al. 1992; Kim et al. 1997; Barichello et al. 2011).

Pathogens may also bind to endothelial cells at or near the TJs (Grab et al. 2004, 2005) and induce the secretion of host proteases that can cleave TJ or AJ proteins. For example, *N. meningitidis* induces specific cleavage of the TJ com-

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ponent occludin through the release of host matrix metalloproteinase 8, resulting in endothelial cell detachment and increased paracellular permeability (Schubert-Unkmeir et al. 2010). A similar strategy is used by *Borrelia*, which was shown to degrade TJ proteins and allow passage of monocytes (Reijerkerk et al. 2006). Some pathogens may adopt a more sophisticated strategy of remodeling the junctional complexes. *E. coli* K1 OmpA initiates a signaling process that leads to the dissociation of β -catenins from cadherins which further results in disruption of barrier integrity (Sukumaran and Prasadarao 2003). The type-IV pili of *N. meningitidis*, on the other hand, stimulates the β 2-adrenoceptor/ β -arrestin signaling pathway in endothelial cells, leading to recruitment of the Par3/Par6/PKC ζ polarity complex and delocalization of junctional proteins which result in anatomical gaps that are used by the bacteria to penetrate into the CNS (Coureuil et al. 2009, 2010). Human immunodeficiency virus (HIV) envelope glycoprotein gp120 has also been shown to increase BBB permeability by interacting with endothelial *trans*-membrane coreceptor CCR5 and CXCR4 (Kannmogne et al. 2007).

Phagocyte-Facilitated Invasion

The use of phagocytic cells for BBB invasion (Trojan horse mechanism) appears to be mainly used by intracellular pathogens that are able to survive and eventually multiply in professional phagocytes. According to this scenario, a pathogen first infects a bone-marrow-derived cell, such as a macrophage, monocyte, or dendritic cell, which may then penetrate the BBB, acting as a shuttle for the microbe. For example, CD11b expressing cells are infected and used by *T. gondii* to breach the BBB (Lachenmaier et al. 2011). Similarly, monocytes or macrophages may be used by *C. neoformans*, *L. monocytogenes*, *M. tuberculosis*, and *Brucella* and *Salmonella* spp for entry into the CNS (Greifenberg et al. 1998; Drevets et al. 2004; Join-Lambert et al. 2005; Charlier et al. 2009). West Nile virus infection of BMEC modulates TJ proteins in endothelial cells and facilitates migra-

tion of virus-infected cells through that space into CNS (Verma et al. 2009). HIV infection of monocytes increases surface expression of LFA-1, an ICAM1 ligand that increases the likelihood of adhesion to CNS endothelial cells (Stent and Crowe 1997). After penetration, these infected monocytes and macrophages can spread the pathogen to microglia, perivascular macrophages, and astrocytes, creating a reservoir for the organism in the CNS. These parenchymal pathogen reservoirs primarily result in microglial activation, leukocyte infiltration into the CNS, and neuronal damage.

Inflammation and BBB Permeability

Finally, host inflammatory factors may also contribute to loss of BBB integrity, resulting in pathogen penetration into the CNS. It was recently shown that polymorphonuclear neutrophils contribute to BBB impermeability loss during the GBS meningitis (Banerjee et al. 2011). Although neutrophils are critically important to fight against bacterial sepsis, depletion of peripheral neutrophils in a murine hematogenous model of GBS meningitis prolonged survival, decreased bacterial CNS load, and decreased BBB leakage in GBS-infected mice (Banerjee et al. 2011). Correspondingly, prevention of leukocyte infiltration into the CNS using anti-CD18 antibody has been shown to improve pneumococcal and HiB meningitis outcomes (Tuomanen et al. 1989; Saez-Llorens et al. 1991).

Different bacterial components initiate inflammatory activation of brain endothelial cells, resulting in secretion of cytokines and chemokines such as TNF- α , IL-1, IL-8, or TGF- β , the levels of which are typically elevated in meningitis patients (Waage et al. 1989; Fida et al. 2006; Nagesh Babu et al. 2008). These cytokines may have an autocrine effect on BMEC, leading to increased expression of host receptors that further promote bacterial penetration. For example, cells stimulated with TNF- α , IL-8, or TGF- β resulted in an increased bacterial uptake in vitro (Cundell et al. 1995; Zhang et al. 2002; Banerjee et al. 2010). OmpA of *E. coli* K1 binds to gp96 glycoprotein on BMEC surface and this interaction in turn increases the expression of

type-1 fimbriae that bind to CD48 (Prasadarao et al. 2003; Khan et al. 2007). Pathogen interaction with BMEC also results in activation of several signal transduction pathways that either leads to actin cytoskeleton rearrangement and subsequent bacterial uptake or proinflammatory chemokine/cytokine secretion and localized inflammation. In the majority of instances actin cytoskeleton rearrangement signaling events involve activation of focal adhesion kinase (FAK) (Prasadarao et al. 1999b; Banerjee et al. 2011), phosphatidylinositol 3-kinase (PI3K), ρ GTPases (Khan et al. 2003) and cytosolic phospholipase A2 (Das et al. 2001) (cPLA₂), and src kinases (Slanina et al. 2010). Additionally, activation of mitogen-activated protein (MAP) kinase signaling pathways promotes proinflammatory cytokine secretion (Sokolova et al. 2004; Banerjee et al. 2010, 2011).

Transcriptional analysis of the BMEC on GBS infection revealed a specific gene-activation program acting to orchestrate neutrophil recruitment (IL-8, CXCL1, CXCL2), activation (IL-6, IL-8), survival (GM-CSF), and extravasation (ICAM-1). This response was dependent on the GBS β -h/c toxin, the transcriptional regulator of virulence, CovR and PilA expression (Doran et al. 2003; Lembo et al. 2010; Banerjee et al. 2011). Additional *in vitro* studies with *S. pneumoniae*, HiB, *S. typhimurium*, *E. coli* K1, *Streptococcus suis*, *L. monocytogenes*, and *N. meningitidis* reveal a similar core BMEC transcriptional response that results in the induction of neutrophil signaling pathways (Wilson and Drevets 1998; Vadeboncoeur et al. 2003; Galanakis et al. 2006; Schubert-Unkmeir et al. 2007; Banerjee et al. 2010; van Sorge et al. 2011). Continued exposure and invasion of the pathogen may result in overactivation of BBB endothelium and lead to increased inflammation that may ultimately compromise BBB integrity.

IN SUMMARY

Microbial interactions with host barriers are key events in invasive infectious diseases. The deciphering of the molecular mechanisms underlying microbial attachment to, invasion of, translocation across, and disruption of host barriers

is not only important in a biomedical perspective but also on a basic perspective. Future studies will hopefully help improve the severe prognosis of invasive infection, and also contribute to a better understanding of the physiology of host barriers.

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