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Mechanisms of microbial traversal of the blood–brain barrier

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Abstract

Central nervous system (CNS) infections continue to be an important cause of morbidity and mortality. Microbial invasion and traversal of the blood–brain barrier is a prerequisite for CNS infections. Pathogens can cross the blood–brain barrier transcellularly, paracellularly and/or in infected phagocytes (the so-called Trojan-horse mechanism). Consequently, pathogens can cause blood–brain barrier dysfunction, including increased permeability, pleocytosis and encephalopathy. A more complete understanding of the microbial–host interactions that are involved in microbial traversal of the blood–brain barrier and the associated barrier dysfunction should help to develop new strategies to prevent CNS infections.

Central nervous system (CNS) infections continue to be an important cause of morbidity and mortality throughout the world. For example, bacterial meningitis is one of the top ten causes of infection-related deaths worldwide[1]. Morbidity and mortality rates vary, however, depending on the age and location of the patient and the causative organism. Patient groups who are at risk of high rates of morbidity and mortality include newborns, the elderly and those living in developing countries, and the infections that have higher rates of morbidity and mortality are those caused by Gram-negative bacilli and Streptococcus pneumoniae[2–5]. A major contributing factor to the lack of preventive measures against CNS infections is our incomplete understanding of their pathogenesis[6,7]. Almost all microorganisms that are pathogenic to humans have the potential to penetrate the CNS, but it is still unclear why a comparatively small number of microbial pathogens account for most cases of CNS infection in humans.

This Review summarizes our current understanding of the mechanisms that are involved in traversal of the blood–brain barrier by selected meningitis-causing microorganisms and the associated blood–brain barrier dysfunction.

DATABASES

Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj

Borrelia burgdorferi | Borrelia turicatae | Candida albicans | Cryptococcus neoformans | Escherichia coli | Haemophilus influenzae | Listeria monocytogenes | Mycobacterium smegmatis | Mycobacterium tuberculosis | Neisseria meningitidis | Plasmodium falciparum | Streptococcus agalactiae | Streptococcus pneumoniae | Streptococcus suis | Toxoplasma gondii | Treponema pallidum

Entrez Protein: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=protein

CNF1 | FbsA | IbeA | IbeB | IL-8 | InlB | Lmb | NadA | OmpA

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

The blood–brain barrier

The blood–brain barrier (FIG. 1) is a structural and functional barrier that is formed by brain microvascular endothelial cells, astrocytes and pericytes. It maintains the neural microenvironment by regulating the passage of molecules into and out of the brain, and protects the brain from any microorganisms and toxins that are circulating in the blood. Brain microvascular endothelial cells have distinctive features, such as tight junctions and low rates of pinocytosis[8–11]. Astrocytes and pericytes help maintain the barrier property of brain microvascular endothelial cells, but their contributions to microbial traversal of the barrier remain incompletely understood. For example, the contributions of astrocytes and pericytes to Escherichia coli translocation of the barrier are minimal. By contrast, astrocytes, together with microglial cells, regulate the recruitment of infiltrating haematogenous cells[12] and might affect the translocation of some microorganisms. In addition, the antimicrobial activities of astrocytes (such as indoleamine 2,3-dioxygenase activity) and the modulation of signal-transduction pathways in brain endothelial cells by pericytes might affect microbial traversal of the barrier[13,14].

Studies of microbial traversal of the blood–brain barrier and the associated barrier dysfunction have become feasible because of the availability of an *in vitro* human brain microvascular endothelial cell (HBMEC) model[8–10,15,16] and *in vivo* models of experimental haematogenous meningitis in infant rats and mice[6,7,17–19].

Microbial traversal of the blood–brain barrier

Pathogens can cross the blood–brain barrier transcellularly, paracellularly and/or by the Trojan-horse mechanism (FIG. 2). Transcellular traversal refers to microbial penetration through barrier cells without any evidence of microorganisms between the cells or of intercellular tight-junction disruption. Paracellular traversal is defined as microbial penetration between barrier cells with and/or without evidence of tight-junction disruption. The Trojan-horse mechanism involves microbial penetration of the barrier cells using transmigration within infected phagocytes.

Transcellular traversal of the blood–brain barrier has been demonstrated for most meningitis-causing bacterial pathogens, including *E. coli*[6,7,17], Streptococcus agalactiae[6,7,20], *S. pneumoniae*[6,7,21], Neisseria meningitidis[6,7,22] and fungal pathogens, such as Candida albicans[23] and Cryptococcus neoformans[24] (BOX 1). Paracellular penetration of the blood–brain barrier has been suggested for the protozoan *Trypanosoma* spp.[25,26] and *Borrelia* spp.[27,28], although these microorganisms have also been shown to traverse the blood–brain barrier by transcellular penetration[25,27,28]. The Trojanhorse mechanism has been suggested for Listeria monocytogenes and Mycobacterium tuberculosis[29], but transcellular penetration of the blood–brain barrier has also been demonstrated for these organisms[6,7,30–32].

The availability of *in vivo* models has enabled us to elucidate some of the mechanisms that are involved in the traversal of the blood–brain barrier by microorganisms. For example, studies have indicated that the primary site of entry into the CNS for circulating *E. coli, S.*

agalactiae and *C. neoformans* is the cerebral vasculature, not the choroid plexus[17,24,33,34]. The interaction between *E. coli* and HBMECs is currently the best-characterized system to study how meningitis-causing pathogens cross the blood–brain barrier[6,7]. In the following sections, the mechanisms that are involved in traversal of the blood–brain barrier by microorganisms that commonly cause CNS infections in humans are summarized, with an emphasis on the similarities and differences between different organisms.

Bacterial invasion and traversal

Several studies in humans and experimental animals point to a relationship between the magnitude of bacteraemia and the development of meningitis following *E. coli, S. agalactiae* and *S. pneumoniae* infections[17,33,35–39]. Other routes of bacterial entry into the CNS include spread from a contiguous source of infection, such as sinusitis or mastoiditis. For example, *S. pneumoniae* has been shown to enter the CNS through a non-haematogenous route in experimental animals after intranasal inoculation and otitis media[39,40]. *E. coli* penetration into the CNS is similar in infant and adult animals with similar levels of bacteraemia[17], which suggests that a threshold level of bacteraemia must be reached before the blood–brain barrier can be breached.

The prevention of bacterial multiplication in the blood could therefore be a potential approach for the prevention of bacterial meningitis. *E. coli* K1 capsular polysaccharides and O-chain lipopolysaccharides have been recognized as crucial surface structures that contribute to resistance to serum- and polymorphonuclear leukocyte-mediated killing[17,41]. Such resistance is a factor in the induction of a high degree of bacteraemia, and studies are underway to identify additional microbial structures that contribute to a high level of bacteraemia and that could be used as protective epitopes in vaccine design[42]. This concept has been successfully applied to Haemophilus influenzae serotype b and *S. pneumoniae*, and protein-conjugated capsular polysaccharide vaccines for these organisms have almost completely eliminated the meningitis that is caused by the vaccine serotypes. For example, introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) led to a substantial reduction in the incidence of pneumococcal meningitis that was caused by PCV7 serotypes in the target population of children aged less than 5 years[43,44]. Use of the vaccine also reduced pneumococcal meningitis among unvaccinated populations through reductions in nasopharyngeal colonization and transmission of vaccine-type pneumococci from vaccinated children[43,44]. One caveat is the apparent increase in the incidence of invasive pneumococcal disease that is caused by non-PCV7 serotypes, such as serotype 19A[45]. By contrast, it is unclear whether the development of meningococcal (*N. meningitidis*) meningitis is associated with a threshold level of bacteraemia, although prevention of invasive meningococcal disease has been achieved by the use of meningococcal group C conjugate vaccines[46].

Recent studies have shown that a high degree of bacteraemia is necessary, but not sufficient, for the development of meningitis and that microbial binding to, and invasion of, HBMECs is a prerequisite for penetration of the blood–brain barrier *in vivo*. This was demonstrated using infant rats with experimental haematogenous *E. coli* meningitis that had been infected

with several isogenic mutants of *E. coli* K1 strain RS218 in which proteins that contribute to HBMEC binding (such as outer-membrane protein A (OmpA)) and invasion (such as IbeA, IbeB, IbeC and cytotoxic necrotizing factor 1 (CNF1)) had been deleted. The deleted strains were significantly less able to traverse the blood–brain barrier than the parental strain despite causing similar levels of bacteraemia (FIG. 3), which indicates that those *E. coli* proteins that contribute to HBMEC binding and invasion are necessary for crossing the blood–brain barrier *in vivo*[6,7,47–51].

Transmission electron microscopy studies have revealed that *E. coli* K1 and *S. agalactiae* invade HBMECs. Internalized bacteria are found within membrane-bound vacuoles and transmigrate HBMECs in an enclosed vacuole without intracellular multiplication and without any change in the integrity of HBMEC monolayers[20,52]. No free bacteria are found in the cytoplasm of HBMECs or between adjacent HBMECs, which confirms that *E. coli* K1 and *S. agalactiae* cross the blood–brain barrier by transcellular penetration.

Pathogenic microorganisms use various strategies to invade host cells, such as endothelial cells. Two of the major mechanisms involve rearrangements of the host cell actin cytoskeleton: the zipper mechanism, which involves the formation of cell protrusions that contact the pathogen, and the trigger mechanism, which involves the formation of membrane ruffles around the pathogen[53,54]. Invasion of HBMECs by meningitis-causing microorganisms also requires rearrangement of the actin cytoskeleton[6,7,20,30,52]. However, the mechanisms that are involved differ between microorganisms[6,7,16,55–57]. Once internalized into membrane-bound vacuoles[6,20,52,57,58], some bacteria can modulate intracellular trafficking to avoid lysosomal fusion.

In vivo, HBMECs are exposed to shear stress through blood flow, which enhances the barrier properties of a HBMEC monolayer[59]. *N. meningitidis* adhesion to brain endothelial cells is inversely affected by shear stress[60], which suggests that cerebral microcirculation is an important attribute in *N. meningitidis* adhesion to the blood–brain barrier.

It is important to note that *E. coli* K1 binding to, and invasion of, endothelial cells has been demonstrated in cells that are derived from the brain, such as HBMECs, but not in cells of non-brain origin, such as human umbilical vein endothelial cells (HUV ECs), human aortic arterial endothelial cells and human iliac vein endothelial cells[61,62], which suggests that the interaction between meningitis-causing *E. coli* K1 and HBMECs is unique. Similarly, Streptococcus suis (a causative agent of meningitis and septicaemia) has been shown to interact with HBMECs but not HUV ECs[63]. It is therefore unclear whether the interactions between meningitis-causing microorganisms and non-brain endothelial cells, such as bone-marrow-derived endothelial cells[64], can be extrapolated to interactions with HBMECs.

Bacterial ligand–receptor interactions

E. coli

As discussed above, microbial binding to and invasion of HBMECs is a prerequisite for successful penetration into the CNS[6,7]. Type 1 fimbriae and OmpA are the two major determinants that contribute to *E. coli* K1 binding to HBMECs[65,66]. FimH (a type 1 fimbrial

adhesin) interacts with CD48 on HBMECs, and the addition of exogenous FimH- or CD48-specific antibodies inhibits *E. coli* K1 binding to HBMECs[67]. It has also been shown that the amino-terminal region and surface-exposed loops of OmpA contribute to binding to HBMECs[68] and that OmpA interacts with HBMECs through *N*-acetylglucosamine (GlcNAc) residues in HBMEC glycoproteins, including gp96 (Refs 66,69). Exogenous OmpA and gp96 (also known as GRP94) and anti-gp96 antibodies inhibit *E. coli* K1 binding to HBMECs, but do not inhibit binding of an OmpA mutant[66,68]. The ability of the OmpA mutant to penetrate into the CNS was significantly reduced compared with the parental *E. coli* K1 strain, and GlcNAc-β1,4-GlcNAc oligomers blocked *E. coli* K1 traversal of the blood–brain barrier in the infant-rat model of experimental haematogenous meningitis[51,62]. These findings indicate that proteins such as FimH and OmpA contribute to the binding of *E. coli* to HBMECs through an interaction with their respective HBMEC receptors (TABLE 1). gp96 is an endoplasmic reticulum paralogue of heat shock protein 90 that is not restricted to the endoplasmic reticulum and has been detected at the surface of HBMECs[66]. gp96 is also a cellular receptor for *L. monocytogenes* Vip, which is involved in infection of the spleen, liver and brain of mice[70]; however, gp96 interactions with OmpA and Vip involve different signal-transduction pathways[70].

A recent study used an *E. coli* DNA microarray to compare an OmpA mutant with its parental *E. coli* K1 strain and revealed that the OmpA mutant expressed significantly fewer *fim* cluster genes[71]. This suggested that the decreased binding of the OmpA mutant might be related to lower expression of type 1 fimbriae. Additional studies are needed to determine whether the *in vitro* and *in vivo* defects of the OmpA mutant are related to the decreased expression of type 1 fimbriae and to understand how the deletion of *ompA* affects type 1 fimbriae expression.

As mentioned earlier, several other *E. coli* determinants that contribute to invasion of HBMECs have been identified[47–50,72]. Recombinant Ibe proteins inhibit *E. coli* K1 invasion of HBMECs[47,73], which suggests that these proteins also contribute to HBMEC invasion through ligand–receptor interactions. This was supported by the identification of a HBMEC receptor protein for IbeA and the fact that a polyclonal antibody raised against this receptor inhibited *E. coli* K1 invasion of HBMECs[74]. In addition, enrichment of IbeA-receptor-expressing HBMECs by fluorescence-activated cell sorting resulted in significantly enhanced invasion by the IbeA-positive *E. coli* K1 strain[74]. CNF1 is a virulence factor that is associated with pathogenic *E. coli* strains that cause extraintestinal infections, including meningitis. CNF1 is an `AB-type toxin`, is composed of an amino-terminal cell-binding domain and a carboxy-terminal catalytic domain that has deaminase activity, and activates Rho GTPases, such as Rho, Rac and Cdc42 (Refs 75,76). CNF1 contributes to *E. coli* K1 invasion of HBMECs *in vitro* and traversal of the blood–brain barrier *in vivo*, and these *in vitro* and *in vivo* effects are dependent on RhoA activation[49]. However, it is unclear how CNF1 enters HBMECs and activates Rho GTPases. It has been suggested that CNF1 is internalized by receptor-mediated endocytosis upon binding to a cell-surface receptor[77]. The HBMEC receptor for CNF1 has been identified as a 37-kDa laminin receptor precursor (37 LRP)[78]. The 37 LRP is a ribosome-associated cytoplasmic protein and is a precursor of the 67-kDa laminin receptor (67 LR). It is unclear how the mature 67 LR is synthesized from

the 37 LRP, but mature 67 LR is present on the cell surface and functions as a membrane receptor for the adhesive basement-membrane protein laminin[79]. It has been shown that the expression levels of 37 LRP and 67 LR are directly correlated with CNF1-mediated *E. coli* K1 invasion of HBMECs[78]. Recent studies have shown that CNF1-expressing *E. coli* K1 upregulates the expression of 67 LR in HBMECs and recruits 67 LR along with focal adhesion kinase (FAK) and paxillin to the site of invading *E. coli* K1 in a CNF1-dependent manner[80]. The 37 LRP–67 LR has been shown to be a cellular target for a range of CNS-infecting microorganisms, including dengue virus, adeno-associated virus and Venezuelan equine encephalitis virus, as well as the prion protein PrP[81–84]. It remains to be determined how these different organisms interact with the same receptor.

S. agalactiae *and* L

monocytogenes. Recent studies have indicated that other meningeal pathogens invade HBMECs through ligand–receptor interactions (TABLE 1). The neonatal meningitis pathogens *S. agalactiae* and *L. monocytogenes* possess several proteins that allow binding to, and invasion of, HBMECs. *S. agalactiae* binding to HBMECs occurs through laminin-binding protein (Lmb), the fibrinogen-binding protein FbsA, pili and invasion-associated gene A (IagA) (through lipoteichoic acid anchoring)[85–88], but it is unclear whether these proteins are unique to cerebrospinal fluid isolates of *S. agalactiae* and whether they contribute to *S. agalactiae* penetration into the CNS.

L. monocytogenes invasion of HBMECs is mediated by internalin B (InlB)[30]. Several HBMEC receptors for InlB have been identified, including gC1q-R (the receptor for the globular head of the complement component C1q) and Met tyrosine kinase[89,90], but their contributions to *L. monocytogenes* invasion of HBMECs are not completely understood. For example, InlB does not compete for the same interaction site on Met as the natural ligand hepatocyte growth factor[91]. *L. monocytogenes* penetration into the CNS has also been attributed to transmigration of *L. monocytogenes*-infected monocytes and myeloid cells across the blood–brain barrier[29], and further studies are needed to determine the major route of *L. monocytogenes* penetration into the CNS. gC1q-R has also been shown to be the HBMEC receptor for Plasmodium falciparum-infected red blood cells (Pf-IRBCs)[92].

S. pneumoniae

S. pneumoniae crosses the blood–brain barrier partly through interactions between cell-wall phosphorylcholine and the platelet-activating-factor (PAF) receptor. This was shown by its partial inhibition of pneumococcal invasion of HBMECs by a PAF-receptor antagonist[21,93] and delayed translocation of pneumococci from the lung to the blood (as well as from the blood to the cerebrospinal fluid) in PAF-receptor-knockout mice[94]. The PAF receptor has also been shown to interact with *H. influenzae* serotype b[95], but the role of the PAF receptor in *H. influenzae* penetration into the CNS remains unclear. Several studies suggested that the role of the PAF receptor differs in *S. pneumoniae* and *H. influenzae* infections. For example, a PAF-receptor antagonist attenuated the `pleocytosis` that was elicited by intracisternal inoculation of *S. pneumoniae* but had no effect on the pleocytosis that was induced by *H. influenzae*[96].

N. meningitidis

N. meningitidis is a human-specific pathogen that interacts with human endothelial and epithelial cells. This interaction involves several microbial structures and proteins, including type IV pili, PilC, *N. meningitidis* adhesin A (NadA) and the Opa and Opc proteins[64,97–99]. However, these observations were derived from *N. meningitidis* interactions with non-brain endothelial cells, such as human bone-marrow-derived endothelial cells, and their relevance to HBMECs remains unclear. Interestingly, the invasion of HBMECs by unencapsulated *N. meningitidis* is mediated by Opc binding to fibronectin, which anchors the bacteria to the integrin $\alpha_5\beta_1$ receptor on the HBMEC surface[22]. However, in the bloodstream, *N. meningitidis* is encapsulated, and the *in vivo* relevance of Opc– fibronectin-mediated binding to integrin is therefore unclear. *N. meningitidis* pili bind to CD46 on HBMECs[98], and their lipooligosaccharides have been shown to contribute to a high level of bacteraemia and subsequent penetration into the CNS[100]; CD46 has also been shown to be the receptor for measles virus, adenovirus and human herpesvirus 6 (Refs 101–103).

M. tuberculosis

Tuberculosis of the CNS is a serious and often fatal disease that affects young children disproportionally. *M. tuberculosis* can cross the blood–brain barrier as a free organism or in infected phagocytes[104] (FIG. 2). One recent study showed that strains of *M. tuberculosis* can invade and traverse HBMECs; invasion was significantly increased for the *M. tuberculosis* strains H37RV and CDC 1551 than for the non-pathogenic Mycobacterium smegmatis, and traversal occurred with *M. tuberculosis* strains but not *M. smegmatis*[31].

DNA microarray analysis identified at least 33 genes that were upregulated by eightfold or more and 147 genes that were downregulated by eightfold or more in *M. tuberculosis* that was associated with HBMECs compared with *M. tuberculosis* that was not associated with HBMECs[31]. Mutants of five *M. tuberculosis* genes were attenuated in their ability to invade and/or survive in the brain[32]. The identification and characterization of *M. tuberculosis* genes that are involved in traversal of the blood–brain barrier should help elucidate the pathogenesis of CNS tuberculosis.

Spirochaetes—Neurosyphilis and neuroborreliosis are prototypic spirochaete infections of the CNS, but the mechanisms that are involved in their traversal of the blood–brain barrier remain unclear. Treponema pallidum can invade through the intercellular junction of aortic endothelial cells[105], which suggests that a mechanism of paracellular penetration of the vascular endothelium occurs, but it is unclear whether a similar mechanism is involved in *T. pallidum* penetration of the blood–brain barrier. A previous study showed that http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj&cmd=Retrieve&dopt=Overview&list_uids=19847 strains traverse HBMECs without any obvious change in the integrity of HBMECs and that this traversal is facilitated by proteases[27]. These findings suggest that the `fibrinolytic system` — linked by an activation cascade — might lead to focal and transient degradation of tight-junction proteins, which would allow *B. burgdorferi* to traverse the blood–brain barrier. Other recent studies revealed that Borrelia turicatae serotype 1, which is defined by the presence of Vsp1 (variable small protein 1), infects the CNS significantly more than the isogenic serotype,

which is defined by the presence of Vsp2, and that Vsp1 binds to HBMECs[28]. *B. burgdorferi* expressing recombinant Vsp1 exhibited an increased interaction with HBMECs. These findings suggest that Vsp1 binding to HBMECs might play a part in borrelial penetration into the CNS.

Receptors and cytokines—Cytokines have been shown to regulate expression of the HBMEC receptors and/or signalling molecules that are involved in the interaction between HBMECs and microorganisms. For example, tumour necrosis factor (TNF)-α treatment of HBMECs resulted in increased invasion of *S. pneumoniae* owing to upregulation of the PAF receptor[21], which is the receptor for *S. pneumoniae* phosphorylcholine. Transforming growth factor (TGF)-β1 treatment of HBMECs significantly increased *E. coli* K1 invasion and traversal of HBMECs[106], whereas treatment with TNF-α and interferon (IFN)-γ had no effect. TGF-β1 has been shown to affect the host cell signal-transduction pathways that are involved in microbial invasion and traversal of HBMECs, such as Rho GTPases and cytosolic phospholipase A2α (cPLA2α), and more work is needed to understand the mechanisms that are associated with cytokine-mediated modulation of microbial–HBMEC interactions.

Further studies are needed to elucidate the ligand–receptor interactions that are involved in microbial traversal of the blood–brain barrier, particularly as the same receptors have been shown to be involved in the pathogenesis of CNS infection by different microorganisms (TABLE 1). It remains speculative whether the expression levels of these receptors dictate the tropism for CNS infection by meningitis-causing microorganisms.

Signal-transduction mechanisms

Recent advances in our understanding of eukaryotic signal-transduction pathways have expedited our understanding of microbial invasion and traversal of the blood–brain barrier, including identification of the signalling molecules that are involved in rearrangements of the host cell actin cytoskeleton. The signal-transduction mechanisms that are involved in actin cytoskeleton rearrangements and HBMEC invasion differ between meningitis-causing microorganisms. For example, actin cytoskeleton rearrangements are a prerequisite for HBMEC invasion by *E. coli* K1, *S. agalactiae* and *L. monocytogenes*. *E. coli* K1 invasion and traversal of the blood–brain barrier involves FAK, paxillin, phosphatidylinositol 3-kinase, Src kinase, Rho GTPases, cPLA2α and 5-lipoxygenase[6,7,49,55,56,66,76,80] (FIG. 4). By contrast, *S. agalactiae* invasion of HBMECs is independent of Src activation, and *L. monocytogenes* invasion of HBMECs is independent of FAK and cPLA2 activation. The microbial–host interactions that contribute to invasion of HBMECs and the relevant signalling mechanisms that are involved have not yet been fully elucidated.

It is important to note that the mechanisms which are involved in entry of HBMECs by meningitis-causing microorganisms differ from those that are involved in the release of cytokines and chemokines in response to these microorganisms. For example, *E. coli* proteins that are involved in binding to and invasion of HBMECs (OmpA and CNF1, respectively) did not affect the release of interleukin-8 (IL-8) from HBMECs[107]. Similar findings were observed for an *S. agalactiae* Lmb mutant, which was defective for the

invasion of HBMECs but induced the same amount of IL-8 as the parental strain[85]. Additionally, *N. meningitidis* invasion of HBMECs involves c-Jun kinases 1 and 2 (JNK1 and JNK2), but the release of IL-6 and IL-8 from HBMECs involves the p38 mitogen-activated protein kinase (MAPK) pathway[108].

Another crucial factor for the development of meningitis is the ability of pathogens to cross the blood–brain barrier as live organisms. As discussed earlier, transmission electron microscopy studies with *E. coli* and *S. agalactiae* (as well as *C. neoformans, C. albicans* and *M. tuberculosis*) revealed that these microorganisms are found within membrane-bound vacuoles in HBMECs and transmigrate HBMECs in an enclosed vacuole. HBMECs possess the complete trafficking machinery that is necessary to deliver microorganism-containing vacuoles to lysosomes[57]. Vacuoles that contained a capsule-deletion mutant of *E. coli* K1 interacted sequentially with early endosomal marker proteins (such as early endosomal auto-antigen 1 and the transferrin receptor) and late endosome and late endosome–lysosomal markers (such as Rab7 and lysosome-associated membrane proteins, respectively), and allowed lysosomal fusion with subsequent degradation inside vacuoles. By contrast, capsule-positive *E. coli* K1-containing vacuoles reached early and late endosomes without fusion with lysosomes, thereby allowing *E. coli* K1 to cross the blood–brain barrier as live bacteria[57]. These findings indicate that the capsule of *E. coli* K1 modulates the maturation of *E. coli* K1-containing vacuoles and prevents fusion with lysosomes, which is necessary for traversal of the blood–brain barrier as live bacteria. Additional studies are needed to understand how the capsule can modulate intracellular vacuolar trafficking and whether similar events occur with other meningitis-causing microorganisms.

Consequences of CNS infection

As discussed above, CNS-infecting microorganisms can induce blood–brain barrier dysfunction by affecting the release and/or expression of cytokines, chemokines and cell-adhesion molecules and/or inducing cytotoxicity and apoptosis in HBMECs, which results in increased blood–brain barrier permeability, pleocytosis and encephalopathy[6]. For example, blockade of pleocytosis by intravenous administration of an anti-CD18 monoclonal antibody that was directed against the β-chain of β2-integrin reduced blood–brain barrier permeability in experimental meningitis that involved intracisternal injection of *S. pneumoniae, N. meningitidis* and *H. influenzae* serotype b[109]. However, intracisternal injection of *S. pneumoniae* and *H. influenzae* serotype b resulted in increased blood–brain barrier permeability in both normal animals and leukopenic animals[110,111]. In addition, pleocytosis was not affected in intercellular adhesion molecule 1-knockout and E- and P-selectin knockout mice with haematogenous meningitis caused by *S. pneumoniae* and *H. influenzae* serotype b[18,19]. These findings indicate that increased blood–brain barrier permeability and pleocytosis might develop independently of each other in bacterial meningitis.

As noted above, meningitis-causing microorganisms such as *E. coli, S. agalactiae, N. meningitidis* and *S. suis* induce the release of cytokines and chemokines from HBMECs, but the mechanisms that are involved differ from those which are involved in their binding to, and invasion of, HBMECs. For example, the release of IL-8 in response to *E. coli* and *S.*

agalactiae infection was independent of the ability of these organisms to bind to and invade HBMECs[85,107]. In addition, the signalling pathways that are involved in the release of IL-8 differed from the pathways that are involved in the invasion of HBMECs by *N. meningitidis* (p38 MAPK and JNK, respectively)[108]. Notably, the release of IL-8 in response to meningitis-causing microorganisms did not occur in non-brain endothelial cells such as HUV ECs[107,112,113], which illustrates how the IL-8 response to meningitis-causing microorganisms is specific to HBMECs.

Another consequence of the microbial interaction with HBMECs is encephalopathy, which has been observed in patients with cerebral malaria and *B. pertussis* infection. The mechanisms that are involved in infection-related encephalopathy remain incompletely understood. For example, Pf-IRBCs have been shown to bind to several molecules on HBMECs[92,114–116] (TABLE 1) and the binding of Pf-IRBCs to HBMECs and/or the sequestration of Pf-IRBCs in the cerebral microvasculature has been shown to cause blood–brain barrier dysfunction, including the release of cytokines and chemokines, increased expression of cell-adhesion molecules, alterations in intercellular proteins and the induction of apoptosis[114–116]. Encephalopathy is a serious complication of *B. pertussis* infection, and a recent study has shown that pertussis toxin induces a transient increase in permeability and transendothelial migration of monocytes in HBMEC monolayers[117], which suggests that pertussis-toxin-mediated blood–brain dysfunction could contribute to encephalopathy.

Conclusions

A major limitation to advances in the prevention and treatment of CNS infection is our incomplete understanding of these diseases and the associated blood–brain barrier dysfunction. Studies of the *in vitro* model of the blood–brain barrier and the *in vivo* animal model of experimental haematogenous meningitis have shed some light on the mechanisms of microbial traversal of the blood–brain barrier, the key step for the development of CNS infections. This traversal is the result of specific microorganism–host interactions and involves host cell signal-transduction pathways that affect host cell actin cytoskeleton rearrangements. It is unclear, however, whether the mechanisms that are involved in the traversal of the blood–brain barrier are similar and/or different in the various pathogens that cause CNS infection, such as bacteria, fungi and parasites (BOXes 1,2; TABLE 1). A more complete knowledge of microbial interactions with the blood–brain barrier might facilitate the development of novel strategies for the prevention and therapy of CNS infections.

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Glossary

Astrocyte	A star-shaped glial cell that supports the tissue of the central nervous system

Pericyte	A cell that is found around capillaries and is related to smooth muscle cells. Pericytes surround the endothelium as single cells. Association with pericytes reduces endothelial apoptosis and stabilizes the vasculature
Pinocytosis	The cellular uptake of extracellular fluid. Involves the formation of caveolae by the cell membrane that pinch off to form vesicles in the cytoplasm
Microglial cell	An immune cell of the central nervous system that is derived from mesodermal precursor cells and could be of haematopoietic lineage
Choroid plexus	A site of production of cerebrospinal fluid in the adult brain that is formed by the invagination of ependymal cells into the ventricles, which then become vascularized
AB-type toxin	A bacterial toxin that modifies target proteins within the cytosol of host cells and is composed of two domains: one that is responsible for the enzymatic activity (A) and one that is responsible for cell-receptor binding (B)
Pleocytosis	The presence of a higher than normal number of cells in the cerebrospinal fluid
Fibrinolytic system	A broad spectrum of proteolytic enzymes that includes the plasminogen activator system and plasmin Plasmin and plasmin activators proteolytically degrade the extracellular matrix
Encephalopathy	Brain dysfunction that is associated with alterations of the neural microenvironment and results from metabolic, toxic, vascular, infectious and/or inflammatory insults

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Box 1

Cryptococcal invasion and traversal of the blood–brain barrier

Cryptococcus neoformans is a common cause of culture-proven meningitis in areas of the world where HIV-1 is endemic, and cryptococcal meningitis is known for its high morbidity and mortality rates[118]. *C. neoformans* is commonly acquired by inhalation. Extrapulmonary dissemination can lead to infection of the bloodstream and subsequent haematogenous dissemination to target organs, most commonly resulting in meningoencephalitis.

Results from experimental mouse models of cryptococcal meningitis following intravenous inoculation, as well as cases of human cryptococcal meningitis, have indicated that *C. neoformans* invasion into the brain follows fungaemia, and that cerebral capillaries, not the choroid plexus, are the portals of entry into the brain[24,119,120]. Cryptococcal invasion of the brain does not require recruitment of host inflammatory cells[24,119,120], which eliminates the possibility that *C. neoformans* traverses the blood–brain barrier using the Trojan-horse mechanism.

A recent study showed that *C. neoformans* strains can enter and traverse human brain microvascular endothelial cells (HBMECs) without any obvious change in HBMEC integrity. Transmission and scanning electron microscopy has revealed that *C. neoformans* induces the formation of microvilli-like protrusions to initiate entry into HBMECs, that *C. neoformans* is found intracellularly in membrane-bound vacuoles and that no free *C. neoformans* cells are found in the HBMEC cytoplasm[24]. These findings indicate that *C. neoformans* uses a transcellular mechanism (FIG. 2a) to enter HBMECs that involves host cell actin cytoskeleton rearrangements.

Several studies have shown that various cryptococcal virulence factors contribute to extrapulmonary dissemination, including the capsular polysaccharide, mannitol, the mating type, melanin, phenotypic switching, phospholipase, prostaglandins and urease[118] (TABLE 1). For example, mutants of *C. neoformans* that lacked laccase were defective in dissemination to extrapulmonary sites in mice following intratracheal inoculation compared with the parental strain, but seemed to be as effective as the parental strain in dissemination to the brain following intravenous inoculation[121]. Similarly, mutants of *C. neoformans* that lacked phospholipase B were defective in extrapulmonary dissemination following intratracheal inoculation compared with the parental strain; the ability of these mutants to disseminate to the brain following intravenous inoculation is unknown[122,123]. A recent report described how cryptococcal urease contributes to dissemination to the central nervous system (CNS) following intratracheal and intravenous inoculation[120], but it is unclear how urease contributes to *C. neoformans* traversal of the blood–brain barrier. The cryptococcal inositolphosphosphingolipid phospholipase C1 gene (*isc1*) has been shown to be important for controlling the dissemination of *C. neoformans* to the brain in mice in which macrophages are depleted[124]. This suggests that macrophage activation is important for preventing fungal dissemination of the Δ*isc1* mutant to the CNS and the development of *C. neoformans* meningoencephalitis. Additional studies are therefore needed to elucidate the microbial–host interactions and associated signal-transduction

pathways that are involved in *C. neoformans* traversal of HBMECs and penetration into the CNS.

Box 2

Parasite invasion and traversal of the blood–brain barrier

The neurological manifestations of sleeping sickness in humans that are caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are attributed to the penetration of the central nervous system by these organisms[25,26]. Bloodstream forms of *T. brucei gambiense* efficiently cross human brain microvascular endothelial cells (HBMECs) by a paracellular route[26]. In experimental rodent models, the parasite can pass through the blood–brain barrier across or between endothelial cells and the vessel basement membranes[25]. The laminin composition of the basement membranes determines whether they are permissive to parasite penetration, and interferon (IFN)-γ has been shown to have an important role in regulating trypanosome trafficking into the brain[25].

Toxoplasma encephalitis is a serious complication of infection with the obligate intracellular parasite Toxoplasma gondii. Stimulation of HBMECs with IFN-γ resulted in the restricted growth of *T. gondii*, which was enhanced in the presence of tumour necrosis factor (TNF)-α. This anti-parasitic activity was due to the induction of indoleamine 2,3-dioxygenase (IDO) in HBMECs by IFN-γ and TNF-α[13]. Repletion of the essential amino acid tryptophan abrogated this inhibition, suggesting that IDO activation and the subsequent degradation of tryptophan is the main mechanism for IFN-γ-mediated and TNF-α-mediated toxoplasmosis. IDO-positive HBMECs can cleave tryptophan to kynurenine and therefore reduce the transport of tryptophan to astrocytes. As IDO is the main effector mechanism in astrocytes against *T. gondii*, reduced tryptophan influx enhances the antimicrobial effect of IDO-positive astrocytes. Additional studies are needed to elucidate the mechanisms that are involved in IDO-mediated toxoplasmosis.

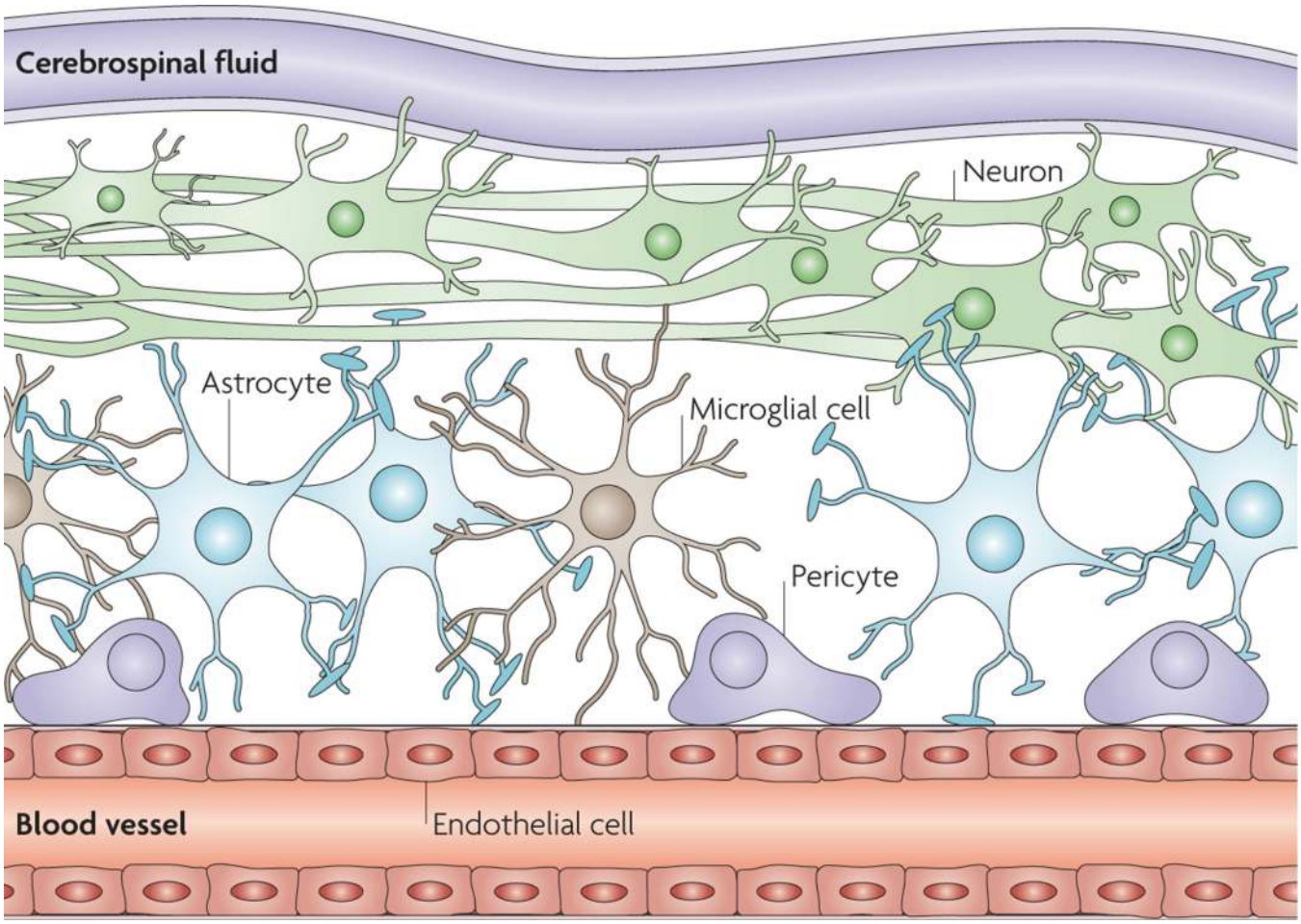

Figure 1. The blood–brain barrier

The blood–brain barrier is formed by brain microvascular endothelial cells, astrocytes and pericytes. It maintains the neural microenvironment by regulating the passage of molecules into and out of the brain, and protects the brain from any microorganisms and toxins that are circulating in the blood.

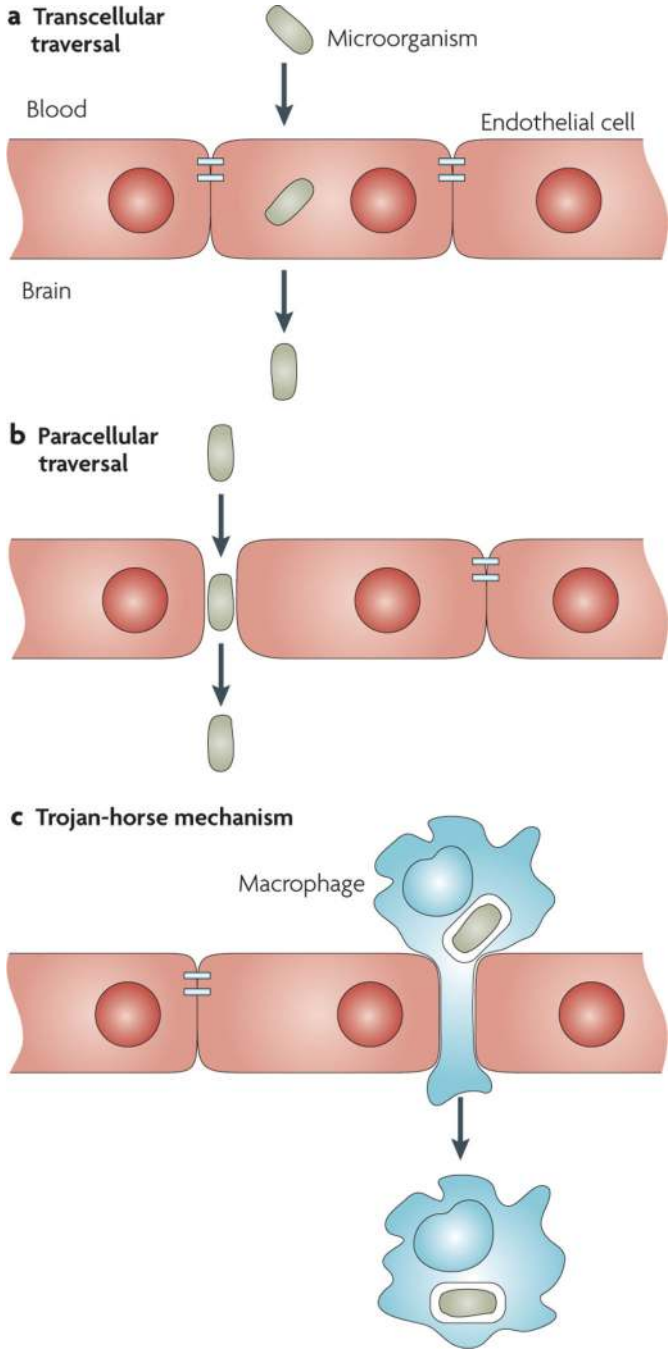

Figure 2. Mechanisms involved in microbial traversal of the blood–brain barrier

Pathogens can cross the blood–brain barrier transcellularly, paracellularly and/or in infected phagocytes (the Trojan-horse mechanism). **a** | In transcellular traversal, the pathogens cross the barrier without any evidence of intercellular tight-junction disruption or detection of microorganisms between cells. **b** | Paracellular traversal involves microbial penetration between barrier cells with and/or without evidence of tight-junction disruption. **c** | The Trojan-horse mechanism involves microbial penetration of the barrier cells using transmigration within infected phagocytes.

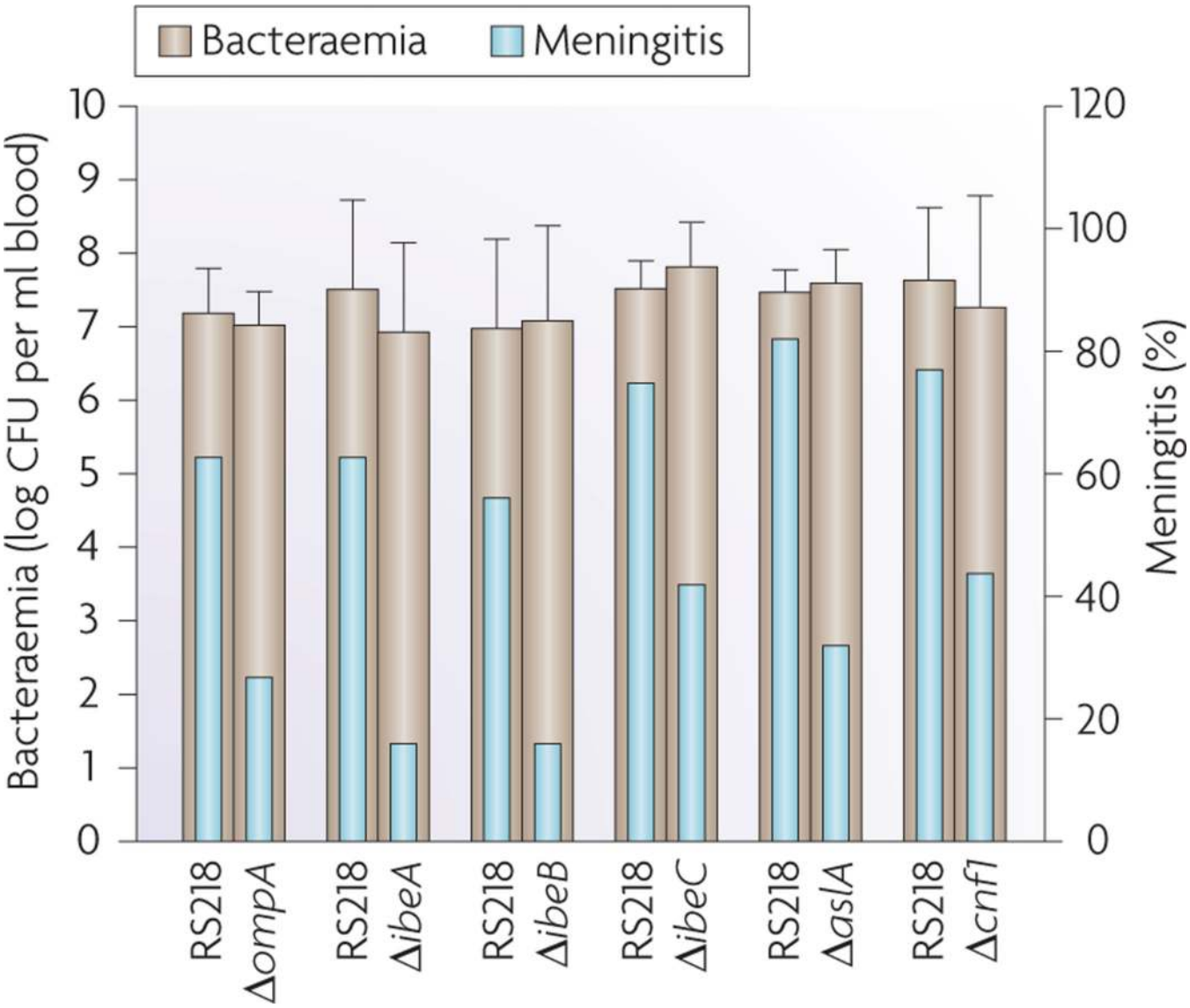

Figure 3. The effect of microbial determinants on *Escherichia coli* meningitis

In infant rats with experimental haematogenous *E. coli* meningitis, isogenic mutants of *E. coli* K1 strain RS218 in which outer-membrane protein A (OmpA), IbeA, IbeB, IbeC, arylsulfatase-like protein (AslA) and cytotoxic necrotizing factor 1 (CNF1) had been deleted were less able to traverse the blood–brain barrier and cause meningitis than the parental strain, despite causing similar levels of bacteraemia. CFU, colony-forming unit.

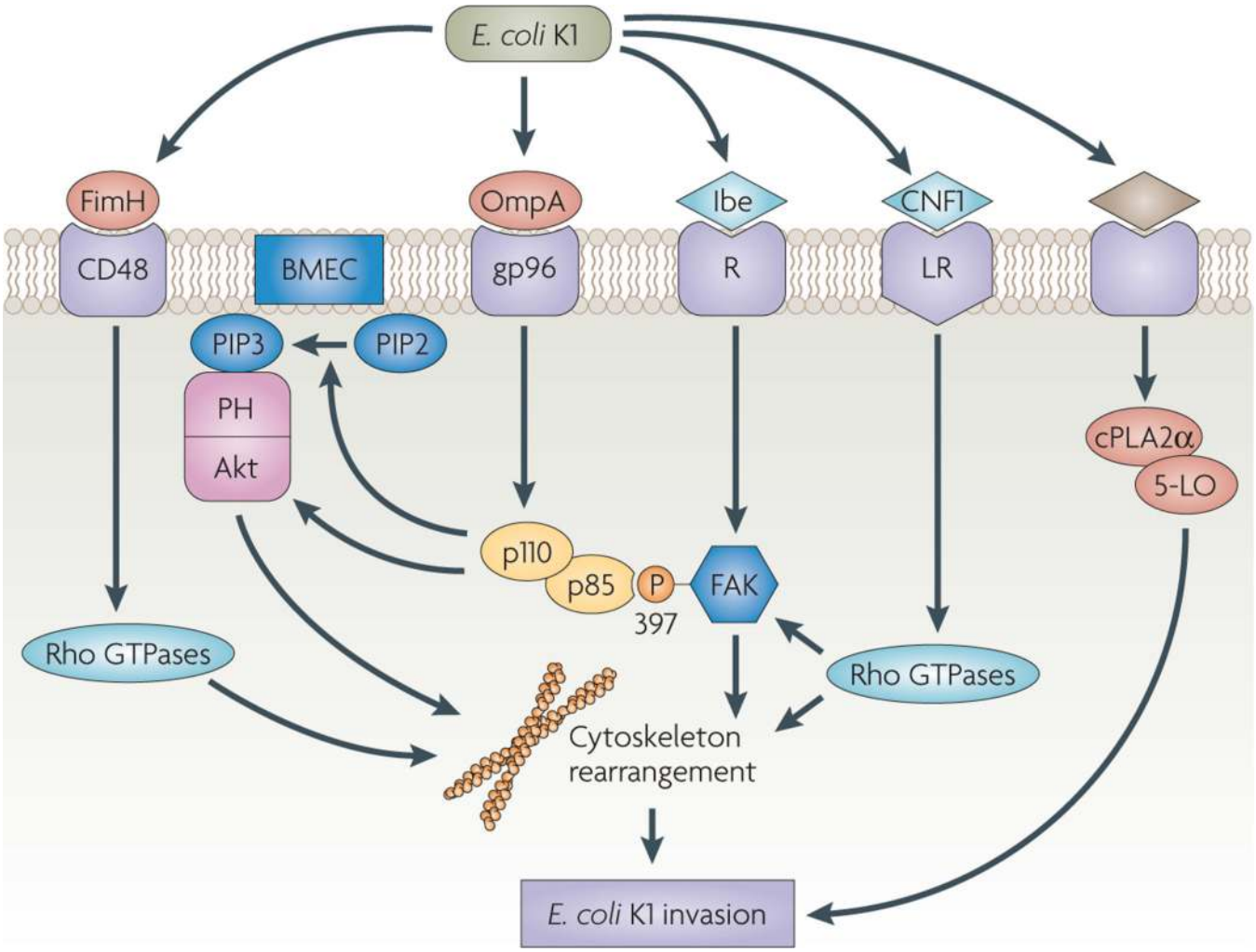

Figure 4. Signalling mechanisms involved in *Escherichia coli* K1-mediated actin cytoskeleton rearrangements and traversal of the blood–brain barrier

E. coli K1 invasion and traversal of the blood–brain barrier occurs as the result of specific bacterial interactions with receptors (CD48, gp96, R and LR) on brain microvascular endothelial cells (BMECs) and involves FAK, paxillin, phosphatidylinositol 3-kinase, Rho GTPases, 5-LO and cPLA2α. 5-LO, 5-lipoxygenase; CNF1, cytotoxic necrotizing factor 1; cPLA2α, cytosolic phospholipase A2α; FAK, focal adhesion kinase; gp96, glycoprotein 96; OmpA, outer-membrane protein A; PH, pleckstrin homology; PIP, phosphatidylinositol phosphate.

Table 1

Ligand–receptor interactions in microbial traversal of the blood–brain barrier

Ligand	Receptor and mechanism	Refs
Escherichia coli		
FimH	CD48; binding to HBMECs	65,67
OmpA	gp96; binding to HBMECs	6,68,69
CNF1	37 LRP–67 LR; invasion of HBMECs	49,78,80
IbeA	45 kDa protein; invasion of HBMECs	47,74
Listeria monocytogenes		
InlB	gClq or Met; invasion of HBMECs	30,89,90
Vip	gp96; penetration into the brain	70
Neisseria meningitidis		
Opc	Fibronectin; invasion of HBMECs through α5β1 integrin	22,97,108
Pili	Possibly CD46; penetration into the brain	97,98
LOS	Unknown; penetration into the brain owing to a high level of bacteraemia	100
Streptococcus pneumoniae		
Phosphorylcholine	PAF receptor; invasion of HBMECs	21,93
Haemophilus influenzae *serotype b*		
Phosphorylcholine	PAF receptor; unknown	95
Streptococcus agalactiae		
Lmb	Laminin; invasion of HBMECs	85
FbsA	Fibrinogen; binding to HBMECs	86
Pili (PilA and PilB)	Unknown; binding to HBMECs	88
iagA	Unknown; binding to HBMECs through LTA anchoring	87
Cryptococcus neoformans		
Capsule, laccase, phospholipase B, urease and Isc1	Unknown; penetration into the brain	24, 118–124
Plasmodium falciparum		
Pf-IRBCs or PfEMP1	Thrombospondin, CD36, ICAM1, gClqR, PECAM or CD31, VCAM1, ELAM1 and chondroitin sulphate A; HBMEC dysfunction	92,113–116

37 LRP–67 LR, 37-kDa laminin receptor precursor–67-kDa laminin receptor; CNF1, cytotoxic necrotizing factor 1; ELAM1, endothelial-leukocyte adhesion molecule 1; gp96, glycoprotein 96; HBMEC, human brain microvascular endothelial cell; *iagA*, invasion-associated gene A; ICAM1, intercellular adhesion molecule 1; InlB, internalin B; Isc1, inositolphosphosphingolipid phospholipase C1; Lmb, laminin-binding protein; LOS, lipooligosaccharide; LTA, lipoteichoic acid; OmpA, outer-membrane protein A; Pf-IRBC, *P. falciparum*-infected red blood cell; PfEMP1, *P. falciparum* erythrocyte membrane protein 1; PAF, platelet-activating factor; PECAM, platelet–endothelial-cell adhesion molecule.