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MINIREVIEW

An insight into the ligand–receptor interactions involved in the translocation of pathogens across blood–brain barrier

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Abstract

Traversal of pathogen across the blood–brain barrier (BBB) is an essential step for central nervous system (CNS) invasion. Pathogen traversal can occur paracellularly, transcellularly, and/or in infected phagocytes (Trojan horse mechanism). To trigger the translocation processes, mainly through paracellular and transcellular ways, interactions between protein molecules of pathogen and BBB are inevitable. Simply, it takes two to tango: both host receptors and pathogen ligands. Underlying molecular basis of BBB translocation of various pathogens has been revealed in the last decade, and a plethora of experimental data on protein–protein interactions has been created. This review compiles these data and should give insights into the ligand–receptor interactions that occur during BBB translocation. Further, it sheds light on cell signaling events triggered in response to ligand–receptor interaction. Understanding of the molecular principles of pathogen–host interactions that are involved in traversal of the BBB should contribute to develop new vaccine and drug strategies to prevent CNS infections.

Introduction

Blood–brain barrier (BBB) is a specialized system, which has a unique role in the protection of the brain from toxic substances in blood and filters harmful compounds from the brain back to the bloodstream. Several pathogens have developed refined and complex mechanisms of BBB disruption and its crossing (by transcellular or paracellular means). The most advanced way of pathogen translocation without mechanical damage of BBB is the so-called Trojan horse mechanism or mimicry of surface ligands on the host cells (like lymphocyte) for traversal across tight junctions. Interestingly, some of the neuroinvasive bacteria are able to express surface receptors for proteases that digest extracellular matrix (ECM) and components of basal membrane. For example, ErpA of *Borrelia* binds to serine protease plasmin that activates matrix metalloproteases and degrades several components (laminin, collagen IV, etc.) of BBB and increases its permeability. Microbial proteins and some nonproteinous factors, like hyaluronic acid or lipooligosaccharide, play a key role in the penetration of BBB. Detailed knowledge of the proteins and nonproteinous compounds, from both pathogen and host sides, associated with BBB translocation, immensely help us to unfold the pathogenesis of brain invasion.

Brain microvascular endothelial cells (BMECs): specialized building blocks of BBB

BBB is a distinctive and protective wall composed of BMECs, astrocytes, basement membrane, and pericytes. Unique property of BBB is primarily determined by the presence of endothelial junctional complexes made up of adherens junctions (AJs) and highly specialized tight junctions (TJs). Apart from the presence of specialized TJs, other unique properties of BBB are (1) absence of fenestrae and reduced level of fluid-phase endocytosis and (2) asymmetrically localized enzymes (Archer & Ravussin, 1994).

AJs are significant for initiating and maintaining endothelial cell–cell contact, while TJs seal the interendothelial cleft forming a continuous blood vessel (Rubin & Stad-

don, 1999). TJs form a circumferential belt that separates apical and basolateral plasma membrane domains (Tsukita *et al.*, 2001) and share biophysical properties with conventional ion channels, including size and charge selectivity, dependency of permeability on ion concentration, anomalous mole-fraction effects, and sensitivity to pH (Tang & Goodenough, 2003). The presence of TJs between BMECs leads to high endothelial electrical resistance and low paracellular permeability.

Transmembrane proteins and cytoplasmic plaque proteins are parts of the TJs and AJs. Former proteins physically integrate with their counterparts on the plasma membrane of contiguous cells. The latter proteins not only link transmembrane TJ/AJ proteins and the actin cytoskeleton but also take part in intracellular signaling (Gonzalez-Mariscal *et al.*, 2003). TJs are composed of the integral transmembranous proteins, occludin, claudins, and junctional adhesion molecules (JAMs), while vascular endothelium cadherin (Ve-cadherin) is the major transmembrane protein of endothelial AJs. Transmembrane proteins of TJs are connected to the actin cytoskeleton by TJ-anchoring proteins, zonula occludens proteins ZO-1, ZO-2, and ZO-3 (Fig. 1).

Infections are quite common, but why do we only see infections of the CNS in rare occasions? One major factor is the special barrier BBB and its building blocks BMECs. BMECs and normal ECs differ from each other in functional and structural terms. Some of these differences are with respect to cytokine and growth-related molecules, stress-related proteins, metabolic enzymes, and signal transduction proteins (Lu *et al.*, 2007). Several TJ proteins, including occludin, claudin-1, claudin-3, claudin-5, claudin-12, JAM-A, JAM-B, JAM-C, endothelial cell-selective adhesion molecule, ZO-1, ZO-2, cingulin, 7H6 antigen, and PAR-3, are expressed differentially in BMECs and peripheral vascular ECs (Nagasawa *et al.*, 2006). For example, claudin-1, claudin-4, claudin-5, claudin-7, and claudin-8 are less abundant in BMECs than in gut ECs; VCAM, ICAM-1, and E-selectin are induced in lower extent than in HUVEC; and the expression of endothelial nitric oxide synthase and ICAM-1 (approximately 30-fold) is lesser than in pulmonary ECs (Panes *et al.*, 1995; Stevens *et al.*, 2001). Occludin and Ve-cadherin are expressed much higher in BMECs compared to non-neuronal ECs (Hirase *et al.*, 1997; Stevens *et al.*, 2001). Similarly, researchers observed high abundance of Lutheran membrane glycoprotein (Shusta *et al.*, 2002), CD46 complement regulator, and autoantigen Ro52 (Shusta *et al.*, 2002)as well as relatively low expression of P-selectin and tissue factor pathway inhibitor on BMECs (Bajaj *et al.*, 1999; Solovey *et al.*, 2004).

It is interesting to note that BMECs express unique cell surface glycoproteins that are not found on other ECs, such as the cerebral cell adhesion molecule, LK48, BBB-specific anion transporter 1, angiogenic factors (vascular endothelial growth factor, follistatin, fibroblast growth factor 1 and 5), and CXC chemokines with Glu-Leu–Arg motifs (epithelial cell-derived neutrophil-activating peptide 78 and growth-regulated oncogene-α) (Grab *et al.*, 2005).

BMECs interact dynamically with neighboring cells, astroglia, pericytes, and microglia that contribute to their unique characteristics. Despite the fact that astrocytes envelop more than 99% of the BBB endothelium, they are not directly involved in the physical properties of BBB (Hawkins & Davis, 2005). Interaction of astrocytes with BMECs induces and modulates development of the unique properties of BBB, that is – reduction in the adhesional and tight junctional gap areas (Tao-Cheng & Brightman, 1988). Previously, it was shown that coculture of BMECs with astrocytes directly affects the maintenance of BBB function and is necessary for its tightness (Tao-Cheng & Brightman, 1988; Holash *et al.*, 1993).

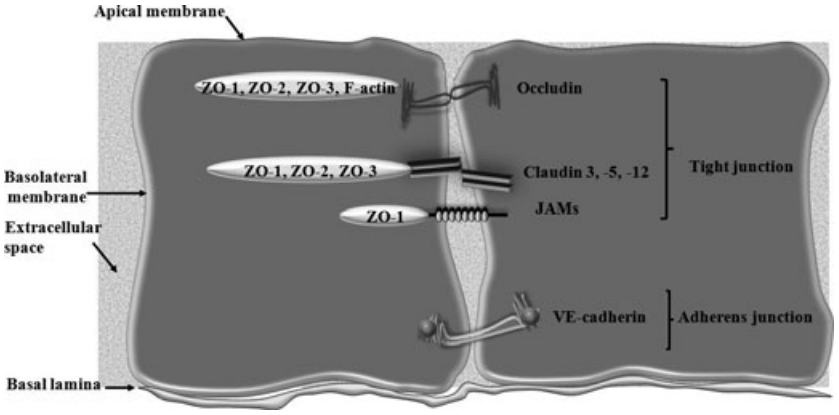

Fig. 1. Tight junctions. Simplified scheme showing the protein composition of brain endothelial tight and AJs. The TJ transmembrane proteins, such as claudin, occludin and JAMs, are responsible for the cellular contact and they are linked to the cytoskeleton via the proteins ZO-1, ZO-2, ZO-3 and F-actin. The AJs, made by Ve-cadherin, are important for the first contact between the ECs.

Crossing of the wall: transcellular vs. paracellular translocation

Only limited numbers of pathogens are capable of penetrating physiologically impermeable biological barriers such as the BBB and the placenta. BMECs seem to be the primary site of pathogen traversal into the CNS. Pathogens may disrupt the BBB and traverse into the CNS via transcellular penetration, paracellular entry, and/or transmigration with infected leukocytes ('Trojan horse' mechanism) (Fig. 2). In the further part of this review, we have focused on transcellular and paracellular traversal of the microorganisms.

Transcellular passage involves penetration of the pathogens through the BMECs. This pathway is initiated by adherence of the pathogen to the ECs leading to the entry of bacterium into the CNS across the BBB using pinocytosis or receptor-mediated mechanisms. Remarkably, some pathogens are able to mimic natural host ligand–receptor interactions that could facilitate interaction between ECs and microorganisms. Transcellular traversal of the BBB has been demonstrated for *Escherichia coli* (Kim, 2000), Group B *Streptococcus* (Nizet *et al.*, 1997), *Listeria monocytogenes* (Greiffenberg *et al.*, 1998), *Mycobacterium tuberculosis* (Jain *et al.*, 2006), *Citrobacter freundii* (Badger *et al.*, 1999), *Haemophilus influenzae* (Orihuela *et al.*, 2009), *Streptococcus pneumoniae* (Ring *et al.*, 1998), and *Candida albicans* (Jong *et al.*, 2001).

The paracellular route is defined as microbial infiltration between barrier cells. This traversal involves loosening of the TJs or disturbing the supporting components of TJs, i.e. basement membrane and glial cells (Tuomanen, 1996). The paracellular transmigration of the BBB has been suggested for the *Trypanosoma* (Grab *et al.*, 2004) and *Treponema pallidum* (Haake & Lovett, 1994). Either the transcellular and/or the paracellular route may serve as possible modes of amoebae entry into the CNS (Khan, 2007). Both routes have also been suggested for *Cryptococcus neoformans* (Chang *et al.*, 2004; Charlier *et al.*, 2005), *Neisseria meningitidis* (Nassif *et al.*, 2002; Coureuil *et al.*, 2009), and Lyme disease pathogen *Borrelia burgdorferi* (Comstock & Thomas, 1991). In addition, phagocyte-facilitated entry into the CNS using Trojan horse mechanisms has been suggested for *L. monocytogenes* and *M. tuberculosis* (Drevets *et al.*, 2004; Join-Lambert *et al.*, 2005).

Transcellular migration mediated by adhesion is described without any evidence of microorganisms between the cells or of intercellular tight-junction destruction. On the other hand, paracellular penetration is characterized with and/or without evidence of tight-junction disruption. Because *in vivo* experiments in humans are difficult or impossible, suitable *in vitro* models of the BBB are essential to understand how pathogen crosses the human BBB. Moreover, *in vivo* inflammatory responses vary greatly according to the specific tissue (vascular bed), stimuli (e.g. pathogen infection, protein ligands, endotoxins, and so on) and the recruited leukocyte subtypes. Given such heterogeneity, it is difficult to make conclusion about the involvement of transcellular vs. paracellular translocation. Further, the majority of *in vivo* reports of transcellular translocation

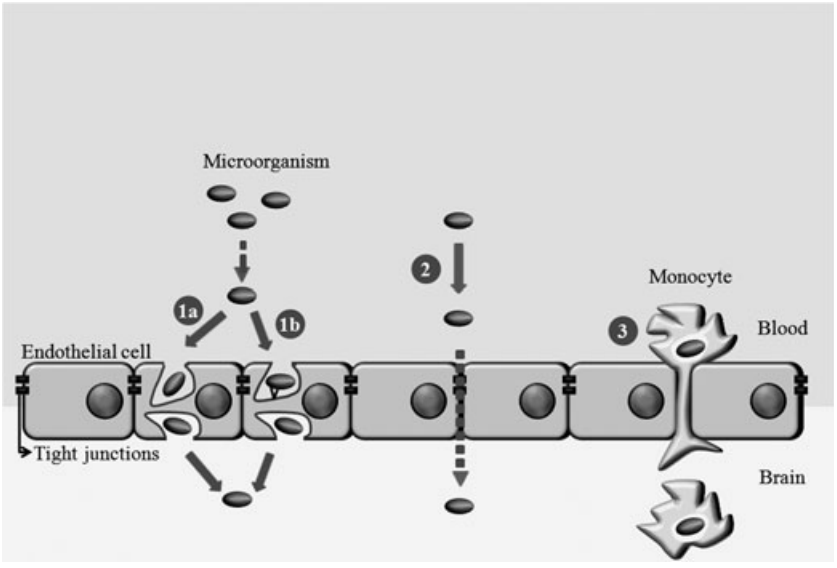

Fig. 2. Cellular pathways through which microorganisms cross the BBB. Pathogens may disrupt the BBB and enter into the CNS through transcellular penetration (1a – pinocytosis, 1b – receptor-mediated mechanism), paracellular entry (2) and/or transmigration with infected leukocytes (Trojan horse mechanism) (3).

have been shown using methods that are often unequivocal (e.g. scanning electron microscopy, transmission electron microscopy, or confocal fluorescence microscopy); however, it is important to note when discussing such reports that some have employed single-section transmission electron microscopy only, which cannot conclusively determine the route of translocation. It is noteworthy that some pathogens may choose the easiest way for BBB translocation. Pathogens and infected leukocytes may preferentially translocate using paracellular route during *in vitro* experiments owing to the fact that cell–cell junctions are not well formed and developed compared to their *in vivo* counterparts (Hoshi & Ushiki, 1999). Moreover, *in vivo* experiments may also depend on the microenvironment of the brain and BMECs that seems to be responsible for the differentiation of the BBB phenotype and astrocytic end-feet cover (Kacem *et al.*, 1998; Rubin & Staddon, 1999; Abbott, 2002), which may influence the route of pathogen translocation.

Proteins of pathogens and their role in BBB translocation

Relatively small number of pathogens is responsible for bacterial meningitis. Group B streptococci (GBS), *L. monocytogenes*, and *S. pneumoniae* account for the most cases of neonatal and early childhood bacterial meningitis (Garges *et al.*, 2006). *Streptococcus pneumoniae*, *N. meningitidis*, and *H. influenzae* type b remain the most common causes of meningitis (Hart & Thomson, 2006), while meningococcus and pneumococcus cause 95% of cases of acute bacterial meningitis in children. Sporadic cases related to *E. coli*, *M. tuberculosis*, *B. burgdorferi*, and *T. pallidum* continue to be important. Fungal meningitis caused by *C. neoformans*, *C. albicans*, and *Histoplasma capsulatum*; and parasitic cerebral infestations caused by *Acanthamoeba*, *Plasmodium falciparum*, *Trypanosoma*, and *Toxoplasma gondii* are sporadic types of meningitis, often observed in patients with immune deficiency.

Bacteria

Epidemiologically important CNS invasive bacteria

GBS

Some GBS molecules, like fibrinogen-binding protein A (Tenenbaum *et al.*, 2005), PilA, PilB (Maisey *et al.*, 2007), laminin-binding protein (Tenenbaum *et al.*, 2007), beta-hemolysin/cytolysin (Doran *et al.*, 2003), serine-rich repeat-1 (van Sorge *et al.*, 2009), and lipoteichoic acid (LTA) (Doran *et al.*, 2005), mediate interaction of the pathogen with BMECs and penetration of the BBB. Many of these GBS ligands are known to bind ECM molecules such as fibronectin, fibrinogen, and laminin, which successively bind host-cell-surface proteins such as integrins. GBS ligands and their receptors on BMECs described earlier are depicted in Table 1. A novel hypervirulent GBS adhesin (HvgA), specific surface-anchored protein, has been reported by French researcher group recently (Tazi *et al.*, 2010). HvgA is essential for the adhesion of bacteria more efficiently to intestinal epithelial cells, choroid plexus epithelial cells, and BMECs. Determination of the structure of HvgA and characterization of its cellular receptor are still under investigation.

β-hemolysin/cytolysin secreted by GBS encourages invasion, conceivably by breaking down host barriers to disclose receptors on the basement membrane, such as laminin (Kim *et al.*, 2005; Maisey *et al.*, 2008). GBS can also bind lysine residues of host plasminogen on its surface to promote the degradation of TJs (Seifert *et al.*, 2003). *iagA* gene also plays prime role in advancing GBS invasion through BBB. This gene encodes an enzyme (homolog of glycosyltransferase) that plays defined roles in the biosynthesis of diglucosyldiacylglycerol, a membrane glycolipid that works as an anchor for LTA (Doran *et al.*, 2005). GBS invasion of BMECs induces actin cytoskeleton rearrangement through phosphorylation of focal adhesion kinase (FAK) and its downstream PI 3-kinase and paxillin, required for its uptake (Shin *et al.*, 2006). Very recent finding has revealed the involvement of another kinase, protein kinase C (PKC) α, in the invasion of GBS across BBB. PKCα activation in BMECs is shown to be dependent on the involvement of cysteinyl leukotrienes, lipoxygenated metabolites of arachidonic acid, and cytosolic phospholipase A (2)α (Maruvada *et al.*, 2011).

Moreover, GBS-infected BMECs induce high levels of activated Rho family members RhoA and Rac1 (Nizet *et al.*, 1997; Shin & Kim, 2006; Shin *et al.*, 2006). Rho-associated pathways could disturb the function of TJs that may lead to increase in BBB permeability.

Listeria monocytogenes

Two pathways of BBB translocation of *Listeria* can be described: (1) direct invasion mediated by proteins internalin B (InlB) and Vip; (2) through the *Listeria*-infected monocytes and myeloid cells via Trojan horse mechanism (Drevets *et al.*, 2004; Join-Lambert *et al.*, 2005).

InlB is a critical protein for the invasion of numerous cell lines, such as HeLa, hepatocytes, and human BMECs. InlB can bind to gC1q-R receptor and Met tyrosine kinase (Braun *et al.*, 2000; Shen *et al.*, 2000). Sequel of the InlB–gC1q-R dyad formation is still unknown; however, interaction between InlB and Met tyrosine kinase

Table 1. Host–pathogen proteins interacting during translocation across endothelial cells

Protein ligand (synonym) [pathogen]	Molecular weight (kDa)/pI	Coding gene (GenBank access)	Length (amino acids)	Chemical nature	General biological functions of the protein, possible way of pathogen translocation	Domain	Start (amino acid)	End (amino acid)	Host receptor
Mannose-binding protein (MBP) [*Acanthamoeba castellanii*]	87.416/4.41	*mbp1* (Q6J288-1)	833	Glycoprotein	Mediates the adhesion of parasites to the host cells, in transcellular/paracellular translocation (Khan, 2007; Khan & Siddiqui, 2009)	CXCX repeat CXCX repeat	317 602	330 615	ND
70-kDa BPBP (plasminogen-binding protein) [*Borrelia burgdorferi*]	59.857/5.64	*bpbp*70 523 (O31313-1)		Lipoprotein	Transport, transporter activity, in paracellular translocation (Comstock & Thomas, 1991; Hu *et al.*, 1997)	SBP bac 5	70	441	Plasminogen
Erp A [*Borrelia burgdorferi*]	19.57/5.84	*erpA*, *erpA5* (Q44781-1)	173	Lipoprotein	Paracellular translocation (Comstock & Thomas, 1991; Brissette *et al.*, 2009)	OspE	41	147	Plasminogen
ErpC [*Borrelia burgdorferi*]	20.2/5.28	*erpC* (Q44790-1)	179	Lipoprotein	Paracellular translocation (Comstock & Thomas, 1991; Brissette *et al.*, 2009)	OspE	41	155	Plasminogen
ErpP (complement regulator-acquiring surface protein 3) [*Borrelia burgdorferi*]	20.6/8.36	*erpP*, *crasp3* (Q9S036-1)	186	Lipoprotein	Paracellular translocation (Comstock & Thomas, 1991; Brissette *et al.*, 2009)	OspE	54	160	Plasminogen
OspA (outer surface protein A) [*Borrelia burgdorferi*]	31/8.77	*ospA* (P0C926-1)	273	Lipoprotein	Transmigration of ECs, in paracellular translocation (Comstock & Thomas, 1991; Rupprecht *et al.*, 2006)	Lipoprotein 1	1	273	Plasminogen
Vsp1 (surface protein VspA) [*Borrelia burgdorferi*]	21.9/8.57	*vspA* (O34000-1)	214	Lipoprotein	Defense response, in paracellular translocation (Comstock & Thomas, 1991; Sethi *et al.*, 2006)	Lipoprotein 6	1	207	Glycosamino-glycans
ALS1 (agglutinin-like protein 1) [*Candida albicans*]	132.6/4.28	*als1* (P46590-1)	1260	Glycoprotein	Adhesion, pathogenesis, in trans-cellular translocation (Fu *et al.*, 1998; Jong *et al.*, 2001)	Repeat domains (1-12)	433	1152	ND
Eno1 (enolase-1, 2-phosphoglycerate dehydratase, 2-phospho-D-glycerate hydrolyase) [*Candida albicans*]	47.2/5.54	*eno1* (P30575-1)	440	Glycolytic enzyme	Glycolysis, entry into host, induction by symbiont of host defense response, in transcellular translocation (Jong *et al.*, 2001; Jong *et al.*, 2003)	Enolase N Enolase C	4 145	135 438	Plasminogen

Table 1. Continued

Protein ligand (synonym) [pathogen]	Molecular weight (kDa)/pI	Coding gene (GenBank access)	Length (amino acids)	Chemical nature	General biological functions of the protein, possible way of pathogen translocation	Domain	Start (amino acid)	End (amino acid)	Host receptor
Isc1 (inositol phosposphingolipid-phospholipase C1) [*Cryptococcus neoformans*]	58.6/6.77	*isc1* (Q1HG89-1)	529	Sphingo-lipid	Ion tolerance, heat stress response, in transcellular translocation (Shea *et al.*, 2006)	P40015	39	341	ND
Urease (urea amidohydrolase, URE1) [*Cryptococcus neoformans*]	90.6/5.65	*ure1* (Q5KCQ6)	833	Amido-hydrolase	Urea metabolic process, increase microenviromental pH, in transcellular translocation (Olszewski *et al.*, 2004)	Urease gamma Urease beta Urease alpha Amidohydro1	1 134 265 391	100 233 385 702	ND
Yps3p [*Cryptococcus neoformans*]	20.4/7.94	*yps3* (Q00950-1)	184	ND	Fungal transition, pathogenicity (Bohse & Woods, 2005)	Calcium-binding EGF domain	49	87	TLR2
AslA (arylsulfatase, aryl-sulfate sulfohydrolase, AS) [*Escherichia coli*]	60.7/5.93	*aslA* (*b3801*, *ECK3793*, *atsA*, *JW3773*, *gppB*, *G616*) (P25549-1)	551	Sulfatase enyzme	Calcium ion binding, sulfor metabolic process, metal ion binding, hydrolase activity, in transcellular translocation (Xie *et al.*, 2004)	Sulfatase	86	516	ND
CNF-1 [*Escherichia coli*]	113/5.26	*cnf1* (Q46962-1)	1014	AB-type toxin	Activate small GTPase of Rho family, in transcellular translocation (Khan *et al.*, 2002)	CNF1	720	1014	40S ribosomal protein SA
FimH [*Escherichia coli*]	30/8	*fimH* (P08191-1)	300	Immuno-globulin-like	Cell adhesion, in transcellular translocation (Khan *et al.*, 2007)	FimH mannose binding Fimbrial C	24 202	171 300	CD48
IbeA (Ibe10) [*Escherichia coli*]	61.6/4.96	*ibeA* (*ibe10*) (Q1R2H6-1)	456	Lipoprotein	Oxidoreductase activity, invasion of BMECs, FAD binding, in transcellular translocation (Huang *et al.*, 1995; Prasadarao *et al.*, 1999; Mendu *et al.*, 2008)	GIDA	18	187	Polypyrimidine tract binding protein-associated splicing factor, vimentin, 45-kDa protein
IbeB (cation efflux system protein CusC, ylcB) [*Escherichia coli*]	50.7/5.93	*ibeB* (P77211-1)	460	Lipoprotein	Pathogenesis, invade BMEC, in transcellular translocation (Prasadarao *et al.*, 1999)	OEP OEP	63 270	247 455	ND
OmpA (outer membrane protein A, outer membrane protein II B0957, Tut, Con, TolG) [*Escherichia coli*]	37.2/6.4	*ompA* (*con*, *tolG*,*tut*) (P0A910-1)	346	Porin	Conjugation, ion transport, phage recognition, in trans-cellular translocation (Wang & Kim, 2002; Prasadarao *et al.*, 2003; Shin *et al.*, 2005)	OmpA-like TD OmpA family	6 222	195 319	Endoplasmin (gp96)

Table 1. Continued

Protein ligand (synonym) [pathogen]	Molecular weight (kDa)/pI	Coding gene (GenBank access)	Length (amino acids)	Chemical nature	General biological functions of the protein, possible way of pathogen translocation	Domain	Start (amino acid)	End (amino acid)	Host receptor
TraJ [*Escherichia coli*]	26.4/8.23	*traJ* (P05837-1)	228	ND	Regulate expression of transfer genes, transcription activator, in transcellular translocation (Will & Frost, 2006)	PAS fold	21	125	ND
YijP [*Escherichia coli*]	68.3/6.91	*yijP* (*b3955 ECK3945*, *JW3927*) (Q8FB99-1)	592	Peptidoglycan	Invade BMEC, in transcellular translocation (Wang *et al.*, 1999)	Dufl 705 Sulfatase	64 252	226 580	ND
Tat [virus HIV]	71.1/9.58	*tat* (P04608-1)	86	Peptide	Apoptosis, interaction host:virus, transcription, in Trojan horse mechanism (Toborek *et al.*, 2005)	Tat	1	65	CD 309
Internalin B (INLB) [*Listeria monocytogenes*]	22.7/6.47	*inlB* (P25147-1)	630	Leucine-rich repeat protein	Mediate entry of bacteria, in transcellular translocation (Greiffenberg *et al.*, 1998; Braun *et al.*, 2000; Shen *et al.*, 2000)	Internalin N LRR 1 LRR adjacent FLG new	3 165 264 350	59 185 321 395	gC1q-R MET
PLB1 (phospholipase B) [*Listeria monocytogenes*]	21.7/4.16	*plb* (E0ACX6)	187	Glycoprotein	Phospholipid catabolic process, in transcellular translocation (Lecuit & Cossart, 2002)				ND
Vip (Lmo0320, proline-rich protein) [*Listeria monocytogenes*]	43.1/4.47	*lmo0320* (E0ACX6)	399	ND	Mediate entry of bacteria, in Trojan horse mechanism (Cabanes *et al.*, 2005)	Pfam-B Gram-positive anchor	1 370	369 399	Endoplasmin (gp96)
GrcC2 (Polyprenyl diphoshate synthase) [*Mycobacterium tuberculosis*]	34.7/5.2	*grcC2* (O05572-1)	325	Polyprenyl synthase	Isoprene biosynthesis, transferase activity, in transcellular translocation (Monroy *et al.*, 2000)	Poyprenyl synthetase	38	276	ND
PPE29 [*Mycobacterium tuberculosis*]	41.4/4.8	*ppe29* (*rv1801Mt1850*) (Q7D7X9-1)	423	ND	Cell invasion, intracellular survival, in transcellular translocation (Jain *et al.*, 2006)	PPE PE PPE C	5 317	162 407	ND
Opc (outer membrane protein) [*Neisseria meningitidis*]	29.9/9.7	*opc*, *opcA* (Q51227-1)	272	ND	Adhesion, invasion, in transcellular translocation (Nassif *et al.*, 1999; Unkmeir *et al.*, 2002; Sokolova *et al.*, 2004)	OpcA	21	264	ECM, integrins heparan sulfate proteoglycan
PilC [*Neisseria meningitidis*]	113.7/9.1	*picC* (P72083-1)	1037	Phase-variable protein	Invasion, in transcellular translocation (Pron *et al.*, 1997; Nassif, 1999; Unkmeir *et al.*, 2002)	PilC	1	1037	CD46

Table 1. Continued

Protein ligand (synonym) [pathogen]	Molecular weight (kDa)/pI	Coding gene (GenBank access)	Length (amino acids)	Chemical nature	General biological functions of the protein, possible way of pathogen translocation	Domain	Start (amino acid)	End (amino acid)	Host receptor
PfEMP1 (erytrocyte membrane protein 1) [*Plasmodium falciparum*]	250.1/5.29	*var* (O97324-1)	2924	Polypeptid	Pathogenesis, cytoadherence, immune evasion, receptor activity (Biswas *et al.*, 2007; Tripathi *et al.*, 2007; Kim, 2008)	Duffy binding PFEMP Duffy binding PFEMP Pfam-B	88 586 947 1459 1741	416 729 1299 1607 2208	TSP1, CD36 Chondroitin Sulfate A gC1q-R Elam 1 VCAM-1 ICAM-1 PECAM
FbsA (fibrinogen-binding protein) [*Streptococcus agalactiae*]	71.1/10.6	*fbsA* (Q84EL6-1)	618	ND	Receptor activity, adherence and invasion to ECs, in trans-cellular translocation (Nizet *et al.*, 1997; Tenenbaum *et al.*, 2005)	Fibrinogen BP Fibrinogen BP	18 252	316 618	Fibrinogen
Lmb (laminin-binding protein) [*Streptococcus agalactiae*]	34.2/8.3	*lmb* (Q8L1H0-1)		Lipoprotein	Cell adhesion, metal ion transport and binding, in transcellular translocation (Nizet *et al.*, 1997; Tenenbaum *et al.*, 2007)	SBP bac 9	8	306	Laminin
PilA (Gbs1478) [*Streptococcus agalactiae*]	101.2/8.1	*gbs1478* (Q8E4C3-1)	901	ND	Adherence, in transcellular translocation (Maisey *et al.*, 2007)	Cna B VWA Pfam-B 8432 Cna B	54 230 416 752	123 414 489 828	ND
PilB (Gbs1477) [*Streptococcus agalactiae*]	73.2/8	*gbs1477* (Q8E4C4-1)	674	ND	Invasion, in transcellular translocation (Nizet *et al.*, 1997; Maisey *et al.*, 2007)	Cna B Gram-positive anchor	471 632	617 669	ND
PAM (plasminogen-binding Group A streptococcal M-like protein) [*Streptococcus pyogenes*]	43.6/8.3	*pam* (*emm*) (P49054-1)	388	M-like protein	Tissue penetration (Berge & Sjobring, 1993)	YSIRK signal VEK-30 (2) Pfam-B 3587	1 90 331	18 119 387	ND
Sag1 (P30) [*Toxoplasma gondii*]	34.7/7.89	*p30* (*sag1*) (P13664-1)	336	GPI-linked protein	Invasion, in Trojan horse mechanism (Fischer *et al.*, 1997; Daubener *et al.*, 2001)	SRS (2)	54	301	ND
FHA (filamentous hemagglutinin protein) [*Bordetella pertusis*]	367.2/8.76	*fhaB2* (Q8VV99-1)	3590	Lectin-like protein	Adherence to epithelium, binding to respiratory cells, in Trojan horse mechanism (Inatsuka *et al.*, 2005)	Haemagg act Pfam-B (4) Fil heamag (5) Pfam-B (6)	71 392 1224 1900	205 999 1787 3589	Fibronectin Factor X C3bi CR3 ICAM-1

ND, not defined.

induces the polymerization of actin, which is necessary for the entry of bacteria into the brain (Cabanes *et al.*, 2005). Previously it was shown that successful invasion of BMECs with *L. monocytogenes* requires not only actin cytoskeleton rearrangements but also Src activation and PI 3-kinase activation (Kim, 2006).

Interestingly, InlB is not only associated with the bacterial surface but also found in culture supernatants of *L. monocytogenes*, indicating that a fraction of this protein is secreted from the bacterial surface (Braun *et al.*, 1997; Jonquieres *et al.*, 1999). Role of this secretory form of InlB in the BBB invasion is yet to be elucidated.

Surface Vip (Lmo0320), a bacterial cell wall-anchored protein, also seems to be an important candidate in late stages of the infectious process. Endoplasmic reticulum resident chaperone Gp96 has been identified as a cellular receptor for Vip (Cabanes *et al.*, 2005). Gp96 is employed in the modulation of the immune response by affecting the cellular trafficking of several molecules, including Toll-like receptors. It is predicted that Vip may not only use Gp96 as a receptor for invasion but may also sequester Gp96 to subvert immunological response. Earlier, researchers predicted the induction and thus the involvement of FAK and PI 3-kinase in the *Listeria* cell invasion as a consequence of Vip–Gp96 binding, as it occurs in *E. coli* invasion. However, later studies showed that *Listeria* interaction with cells does not seem to induce FAK activation for cytoskeletal rearrangements. Similarly, no involvement of the Vip in the increase in tyrosine phosphorylation of protein associated with p85α or Gp96 has been reported elsewhere (Cabanes *et al.*, 2005). Thus, the role of Vip–Gp96 interaction in the *Listeria* cell entry might be through other signal transduction events associated with Gp96 responses that remain to be elucidated.

Another mechanism of BBB translocation, a Trojan horse, needs internalization/phagocytosis of the pathogen by monocytes wherein InlA and InlB play a crucial role. These internalins and P60 protein bind specific receptors (like complement receptor) on phagocytic cells and trigger the internalization of bacteria through a variety of opsonin-dependent and opsonin-independent mechanisms. Internalization allows persistence in a shielded niche, concealed from circulating antibodies. *Listeria*, in its intracellular form, stimulates NF-κB and secretion of cytokines IL-1α, IL-1β, IL-6, and TNF-α in phagocytes. *Listeria*-infected monocytes further upregulate E-selectin, ICAM-1, P-selectin, and VCAM-1, which leads to the adherence to BMECs. The mechanism for this endothelial activation involves listeriolysin O-dependent triggering of NF-κB nuclear translocation in cerebral vessels (Kayal *et al.*, 1999). Infected phagocytes may adhere to endothelium and thus bacteria can invade ECs by cell-to-cell spread in an *hly*- and *actA*-dependent process (Greiffenberg *et al.*, 1998; Drevets, 1999). Infected phagocytes then cross the endothelial barrier, and infection can spread to the brain parenchyma cells or subarachnoid space and ventricles (Drevets & Leenen, 2000). As an alternative to adhering to and infecting the endothelium, infected phagocytes could transmigrate and enter the brain tissue. In this case, bacteria contained within phagocytes could spread to cells such as neurons and microglia (Dramsi *et al.*, 1998).

Streptococcus pneumoniae, *N. meningitidis*, and *H. influenzae* type b

Interestingly, pneumococcus, meningococcus, and *H. influenzae* adhere to the BMECs via 37/67-kDa laminin receptor (LR). This binding occurs via pneumococcal CbpA, meningococcal PilQ and PorA, and OmpP2 of *H. influenzae* (Orihuela *et al.*, 2009). It is remarkable that these pathogens use the same strategy for targeting BBB receptor. Invasion of human ECs in pneumococcus and *H. influenzae* infection is promoted by cytokine activation, which increases the amount of surface-expressed platelet-activating factor receptor (PAFr), which in turn binds the phosphorylcholine (Cundell *et al.*, 1995; Swords *et al.*, 2001). Binding of bacterial phosphorylcholine to PAFr leads to the activation of β-arrestin–mediated endocytosis of the bacteria into BMECs (Radin *et al.*, 2005). A novel candidate ligand that involves in the interaction of pneumococcus and BMEC has been revealed recently. Neuraminidase A (NanA) of pneumococcus mediates BBB activation via laminin G-like lectin-binding domain. NanA induces bacterial uptake, which emphasizes a novel role of neuraminidase in the pathogenesis of pneumococcal meningitis (Banerjee *et al.*, 2010). In addition, pneumolysin, a protein secreted by *S. pneumoniae*, forms transmembrane pores in BMECs, which affects BBB integrity and facilitates brain infection (Zysk *et al.*, 2001).

An important role in meningococcal invasion of the BBB has also been proposed for outer membrane protein Opc and pili type IV proteins PilC (Pron *et al.*, 1997; Nassif, 2000). Opc binds to fibronectin and vitronectin, which anchors the bacterium to the endothelial αVβ3-integrin (the vitronectin receptor) and α5β1-integrin (the fibronectin receptor) (Unkmeir *et al.*, 2002; Sa *et al.*, 2010). Taken together, Opc mediates interactions with host-cell integrins by a bridging mechanism utilizing RGD-bearing serum proteins (arginine–glycine–aspartic acid, RGD motif), which leads to the activation of cytoskeleton-linked pathways (Virji *et al.*, 1994). Opc-mediated interaction induces c-Jun N-terminal kinases 1 and 2 (JNK1/2) and p38 mitogen-activated protein kinases (MAPK) in BMECs. JNK activation is followed by the uptake of the bacterium, while p38 MAPK cascade initiates cytokine release (Sokolova *et al.*, 2004).

Pili type IV proteins of *Neisseria* bind to the host cell receptor CD46 (Kallstrom *et al.*, 1997; Kirchner *et al.*, 2005). The involvement of pili in adhesion to ECs contributes to the formation of microvilli-like cell membrane protrusions underneath bacterial colonies, which help the bacterium to form microcolonies on the EC surface and to destabilize cellular junctions (Mairey *et al.*, 2006; Coureuil *et al.*, 2009). The construction of these protrusions come from the polymerization of cortical actin involved in the clustering of integral membrane proteins, such as ICAM-1, CD44, and the tyrosine kinase receptor ERBB2, as well as ezrin and moesin. The clustering and activation of ERBB2 by homodimerization is responsible for the downstream activation of Src tyrosine kinase activity and for the tyrosine phosphorylation of cortactin. Cortactin is an actin-binding protein that contributes to polymerization and remodeling of the cortical actin cytoskeleton (Weed & Parsons, 2001; Selbach & Backert, 2005). Components such as Rho GTPases and cell division cycle protein 42 and activation of PI 3-kinase–RAC1 –GTPase signaling pathway are essential for cortical actin polymerization induced by *N. meningitidis* (Eugene *et al.*, 2002; Lambotin *et al.*, 2005). In the end, *N. meningitidis* was also found to recruit the endothelial polarity complex, formed by partitioning-defective protein 3 (PAR3), PAR6, protein kinase Cζ (PKCζ), components of both AJs (Ve-cadherin, p120 catenin, α-catenin and β-catenin) and TJs (claudin-5 and ZO-1) at the sites of bacterial adhesion and thereby reducing the integrity of the brain endothelial junctions (Coureuil *et al.*, 2009). The above-mentioned sequence of events leading to cell–cell disruption by rearrangement of the intercellular junction molecules precedes cleavage of occludin by MMP-8 (Schubert-Unkmeir *et al.*, 2010). This could allow paracellular transport of pathogen across the BBB.

Further, it is noteworthy that complex signaling events induced by pathogen are analogous to those initiated by leukocyte adhesion on ECs enabling strong adhesion and extravasation of leukocytes through paracellular as well as transcellular routes.

Sporadically occurring CNS invasive bacteria

Escherichia coli

Several *E. coli* structures contribute to binding and invasion of BMECs, such as type 1 fimbriae (FimH), outer membrane protein A (OmpA), Ibe proteins (IbeA and IbeB), YijP, AslA, and cytotoxic necrotizing factor 1 (CNF-1). AslA protein, member of the arylsulfatase enzyme family, cleaves sulfate esters and plays a role in the penetration of BBB (Hoffman *et al.*, 2000). IbeA interacts with the specific receptor vimentin, which causes the activation of FAK and paxillin leading to cytoskeletal rearrangements and thus allowing *E. coli* to cross the endothelial monolayer (Chi *et al.*, 2010). IbeB and OmpA interact with different receptors on BMECs, yet the effects of these interactions are additive. OmpA interacts with glycoprotein gp96 of BMECs via N-glucosamine epitopes and leads to the FAK-dependent invasion of bacteria, as described earlier (Khan *et al.*, 2002; Wang & Kim, 2002).

CNF-1 is a dermonecrotic, high–molecular weight protein that activates Rho GTPases by deamidation of glutamine, converting it into glutamic acid, inhibiting GTP-hydrolyzing activity and constitutive activation of Rho and ezrin. Ezrin links F-actin filaments to the plasma membrane proteins and induces the formation of microvilli-like membrane protrusions (Khan *et al.*, 2002; Xie *et al.*, 2004). These protrusions are exploited by bacteria for BBB invasion.

FimH, a major adhesion protein, has lectin-like activity with high affinity to mannose residues. Mannose-recognition domain of FimH induces Ca^{2+} surge in BMECs which leads to actin cytoskeleton rearrangements. CD48 seems to be a mannose-containing receptor for FimH. The mannose-insensitive FimH binding, mediated through ATP synthase β-subunit, may also contribute to *E. coli* binding to BMECs to penetrate into CNS (Shin & Kim, 2010). FimH also activates Ras homolog gene family member A (RhoA), and this may contribute to bacterial entry (Khan *et al.*, 2007).

Novel *E. coli* ligand, yet uncharacterized, seems to be involved in vascular endothelial growth factor receptor 1 (VEGFR1)–dependent invasion of BMECs. Stimulation by *E. coli* ligand promotes the physical association between VEGFR1 and p85 subunit of PI-3 kinase. VEGFR1 is necessary for PI-3 kinase/Akt activation and actin cytoskeleton rearrangements (Zhao *et al.*, 2010).

Borrelia burgdorferi

Variable small protein 1 (Vsp1) of *Borrelia turicatae* has been shown to bind to the BMECs (Sethi *et al.*, 2006) and predicted to be involved in the passage of *Borrelia* through BBB. In addition, *B. burgdorferi* is able to adhere to proteoglycans in the ECM of the peripheral nerves and ECs (Leong *et al.*, 1998). It is a well-known fact that *Borrelia* can bind plasminogen and promotes degradation of the ECM (Coleman *et al.*, 1997). On the other hand, fibrinolytic system also initiates other proteases, including matrix metalloproteinases (MMPs), which are predicted to be essential for borrelial invasion into the brain (Grab *et al.*, 2005). OspA and OspE/F-related proteins (ErpP, ErpA, and ErpC) are crucial for the binding of plasminogen (Comstock & Thomas, 1991; Lahteenmaki *et al.*, 2001; Brissette *et al.*, 2009). *Borrelia* is also capable of stimulat-

ing adhesion proteins like E-selectin, ICAM-1, VCAM-1, etc. (Coburn *et al.*, 1993, 1998; Ebnet *et al.*, 1997), which renders host cells more susceptible to pathogen invasion (Table 1).

Treponema pallidum

The pathogenic *T. pallidum* adheres to the vascular endothelium and readily penetrates surrounding tissues. Lee and coworkers (Lee *et al.*, 2003) have also proposed a role of fibronectin in the mediation of the attachment of *T. pallidum* to host cells. It is also predicted that *T. pallidum* interacts with laminin (laminin-1, laminin-2, laminin-4, laminin-8, and laminin-10) with its molecule Tp0751 and may promote tissue invasion. It was also shown that 10 amino acids between the positions 98–101, 127–128, and 182–185 in Tp0751 are critical for the laminin attachment (Cameron, 2003). Furthermore, *T. pallidum* induces the expression of ICAM-1 and procoagulant activity on the surface of HUVEC. ICAM-1 expression in HUVEC is promoted by a 47-kDa integral membrane lipoprotein of *T. pallidum* (Riley *et al.*, 1992). Forty-seven-kilodalton lipoprotein also induces other adhesion molecules like VCAM-1 and E-selectin and promotes the adherence of T lymphocytes to ECs (Lee *et al.*, 2000). This indicates an important role of spirochete membrane lipoproteins in EC activation and translocation.

Other neuroinvasive bacteria

CNS invasion of bacteria described below is rare, yet it is important to know in brief their modes of BBB translocation.

The zonula occludens toxin produced by *Vibrio cholerae* causes TJ disruption by triggering signaling processes, like phospholipase C and PKCα activation, and actin polymerization. Zonula occludens toxin along with its human homolog zonulin is able to bind surface receptor in the brain (Lu *et al.*, 2000). Direct influence of bacterial toxin on the BBB alone or in combination with host's inflammatory mediators such as nitric oxide, TNF-α, and IL-1 enhances BBB permeability (Mun-Bryce & Rosenberg, 1998). Increased permeability of BBB by pertussis toxin (PT) of *Bordetella pertussis* is recently reported. Authors speculate the role of PT-dependent hyperpermeability that may facilitate entry of *Bordetella* and other coinfections like *E. coli* via 'Trojan horse' mechanism (Seidel *et al.*, 2011). Subunits encoded by *ptx* and other associated genes form PT secretion system. In the last years, increasing attention has been given to this secretion complex to unfold its role not only in the translocation of *Bordetella*, but also in coinfections. Inversely, role of type III secretion system in the translocation of *Salmonella enterica* serovar Typhimurium has been ruled out recently (van Sorge *et al.*, 2011). BMEC invasion by *Salmonella* seems to be dependent on actin cytoskeleton rearrangements only.

Earlier, we have described that bacteria exploit host fibrinolytic components, plasminogen/plasmin, to increase the permeability of BBB. Plasmin-binding protein (PAM) of *Streptococcus pyogenes* attracts plasminogen, which is successively activated by streptokinase, and this active plasminogen remained bound to streptococcal surface (Berge & Sjobring, 1993). Plasminogen is also exploited by *M. tuberculosis* with the help of various plasminogen-binding and activating molecules like 30-kDa, 60-kDa, and 66-kDa cell wall proteins (Monroy *et al.*, 2000) (Table 1). Some bacteria alter the expression of TJ proteins and thus the permeability of the BBB. This mechanism is described for *Chlamydiophila pneumoniae*. Chlamydiophilae increase the expression of the zonula adherens proteins (beta-catenin, N-cadherin, and Ve-cadherin) and decrease expression of the tight junctional protein occludin. These events may lead to junctional alterations and BBB breakdown (MacIntyre *et al.*, 2002). In contrast to other meningitis-causing bacteria, interestingly, *C. freundii* is able to multiply within human BMECs. This may be a mechanism whereby *C. freundii* traverses the BBB via transcellular route (Huang *et al.*, 2000).

Fungi

Like *Borrelia*, *S. pyogenes*, and *M. tuberculosis*, *C. albicans* also exploits host plasminogen system. It is shown that interaction between *Candida* enolase and plasminogen results in the invasion and traversal through BMECs (Jong *et al.*, 2003) (Table 1). Fibronectin, laminin, and vitronectin have also been shown to participate in the adherence of *C. albicans* to ECM (Klotz & Smith, 1991; Forsyth *et al.*, 1998; Spreghini *et al.*, 1999). Previously, it was demonstrated that expression of the agglutinin-like ALS1 protein is responsible for the adherence to HUVEC and epithelial cells (Fu *et al.*, 1998). Although HUVEC and BMECs differ in some structural and functional aspects, it is tempting to speculate whether ALS1 protein may also contribute to the invasion of BMECs, and further studies in this aspect are needed. Other potential candidate molecules that may involve in the BMEC transcytosis can be secretory aspartyl proteinases *SAP1-SAP9* of *C. albicans* (Ibrahim *et al.*, 1998; Naglik *et al.*, 1999).

Cryptococcus neoformans can traverse BMECs without any obvious change in their integrity. Transmission and scanning electron microscopy has revealed that *C. neoformans* induces the formation of microvilli-like protrusions to initiate entry into BMECs. These findings indicate that *C. neoformans* uses a transcellular mechanism (Chang

et al., 2004). Very recent finding (Huang *et al.*, 2011) unfolds cryptococcal invasion via lipid raft – endocytic pathway. CD44 molecules from lipid rafts can directly interact with hyaluronic acid of *C. neoformans*. The lipid raft molecule, ganglioside GM1, colocalizes with CD44 on the plasma membrane to which *C. neoformans* can adhere. Upon adhesion, cryptococci are internalized into the BMECs along with GM1 through vesicular structures. Apart from CD44, this endocytosis process is dependent on microtubule cytoskeleton and intracellular kinase-DYRK3 (dual-specificity tyrosine-phosphorylation-regulated kinase 3).

Histoplasma capsulatum is able to invade CNS via surface protein Yps3p. This protein is expressed as secretory protein in infected cells and may have a regulatory role in fungal transition and pathogenicity. Yps3p triggers TLR2 signaling and leads to the activation of NF-κB in microglial cells (Bohse & Woods, 2005) (Table 1).

Parasites

Plasmodium falciparum erythrocyte membrane protein (PfEMP-1) mediates endothelial binding and affects barrier integrity. PfEMP-1 binds to ICAM-1, CD36, chondroitin sulfate, and other trypsin-sensitive binding determinants (Tripathi *et al.*, 2007). Pathogen matures in parasitized red blood cells, which get attached to BMECs. This process is mediated by specific molecular adhesive events. This binding is not solely static but can be a rolling interaction, similar to the early rolling that allows subsequent leukocyte tethering to ECs during physiological responses to inflammatory stimuli (Cooke *et al.*, 1994).

The ability of trypanosomes to invade the brain and induce an inflammatory reaction is well recognized. Process of trypanosomal traversal across the human BBB requires the participation of a PAR-2-mediated calcium signaling pathway. Work of Grab and his colleagues (Grab *et al.*, 2004) shows that *Trypanosoma* translocates BBB by generating Ca^{2+} activation signals by parasite cysteine proteases. Trypanosomal cathepsin (brucipain) can initiate BBB translocation and increases vascular permeability by interaction with host G protein-coupled receptors (Abdulla *et al.*, 2008).

The mechanism by which *Acanthamoeba* transmigrates the BBB is the most complex and may involve both pathogen (adhesins, proteases and phospholipases) and host factors (IL-β, IL-α, TNF-α, IFN-γ, and host cell apoptosis). The overall consequence of these factors is increased permeability and/or apoptosis of the BMECs, which encourages BBB disruptions leading to CNS invasion (Khan, 2008). The adhesion to BMECs appears to be an important step in invasion of *Acanthamoeba* in the BBB, as nonpathogenic environmental isolates show minimal binding to BMECs (Alsam *et al.*, 2003). Phospholipases influence the release of arachidonic acid from the cell surface (Dieter *et al.*, 2002). Arachidonic acid is a prostaglandin precursor that increases BBB vascular permeability and nitric oxide production in BMECs (Harris *et al.*, 2002). Similarly, extracellular serine proteases and/or mannose-binding protein cause redistribution/alteration of TJ proteins, such as ZO-1 and occludin (Khan & Siddiqui, 2009) (Table 1).

In addition, it is reported that during the process of adhesion to BMECs, *Acanthamoeba* upregulates the production of proteases (Alsam *et al.*, 2005). *Acanthamoeba* also induces the activation of Rho-associated intracellular signaling cascades. RhoA regulates myosin light-chain phosphorylation causing a change in structure and rearrangement of ZO-1 and occludin, which in turn causes an increase in BBB permeability (Shen *et al.*, 2006; Khan & Siddiqui, 2009). Sissons and coworkers have shown that PI 3-kinase plays an important role in the amoeba-mediated BMECs apoptosis (Alsam *et al.*, 2005). Moreover, *Acanthamoeba* has been shown to be able to stimulate the expression of *GADD45A* and *p130Rb* genes, which are associated with cell cycle arrest (Sissons *et al.*, 2004). These events are sufficient for BMEC dysfunction.

There are two possible routes by which *T. gondii* may cross the BBB. It may enter into the CNS through infected cells, such as monocytes and macrophages. *Toxoplasma gondii* modulates gene expression (E-selectin and P-selectin, ICAM-1, toll-like receptor 4, etc.) of BMECs to promote its own migration across the BBB in a 'Trojan horse' manner through the cells expressing CD11b either with or without CD11c (Lachenmaier *et al.*, 2011). Besides, the parasites may infect and destroy ECs (Daubener *et al.*, 2001). Surface antigen 1 (SAG1), major tachyzoite surface molecule, has been proposed as a ligand that mediates BMEC invasion (Gay-Andrieu *et al.*, 1999).

Viruses

Viruses probably account for the most cases of meningitis. The commonest viruses causing meningitis, enteroviruses, flaviviruses, and lentiviruses, in immunocompromised infants lead to substantial neurological complications and mortality. Remaining viral meningitis and CNS infections are caused by herpes simplex virus (HSV) and flaviviruses, although mumps infection is re-emerging. Viruses enter the CNS through several mechanisms (1) by hematogenous spread and direct traversal through BBB (enteroviruses), (2) virus particles are carried across infected leukocytes (mumps, measles, or herpes viruses) and (3) axonal flow through peripheral and cranial nerves (polio, rabies, and HSV) (Chadwick, 2005).

The penetration of HIV into the CNS through neurons by axonal flow, as occurs with herpes virus and rabies virus, is less probable because the CD4 receptor, the main receptor that enables HIV to infect the cell, is absent on neurons (Gendelman *et al.*, 1998). The Trojan horse mechanism of transport across BBB is considered to play a crucial role in the pathogenesis of viral meningitis in the late phase of AIDS. This model has gained rapid favor; however, recent studies change this model by showing that the vast majority of virions transmitted in trans originate from the plasma membrane rather than from intracellular vesicles (Cavrois *et al.*, 2008). The mechanisms of BBB disruption during retroviral-associated pathologies are not fully understood yet. Most of the studies are focused on the effect of soluble molecules secreted by infected lymphocytes on BBB functions and intercellular TJ organization. In case of HIV infection, the viral protein Tat has been shown to induce cell apoptosis and disruption of the TJs (Andras *et al.*, 2003). In short, Tat-mediated downregulation of claudin-5 plays an important role in altered integrity of BMEC that aids viral transport across BBB (Andras *et al.*, 2005).

West Nile virus (WNV)-associated encephalitis is characterized by disruption of the BBB, enhanced infiltration of immune cells into the CNS, microglial activation, inflammation, and eventual loss of neurons (Glass *et al.*, 2005; Sitati *et al.*, 2007). WNV gains entry into the CNS via the transcellular pathway, without compromising the BBB integrity instead of paracellular pathway (Verma *et al.*, 2009).

Tick-borne encephalitis (TBE) virus causes severe encephalitis with serious sequel in humans. The mechanisms underlying how TBEV gains access to the CNS are not completely elucidated. There are several hypothetical routes for TBEV traversal across BBB. These include (i) cytokine-mediated BBB breakdown, (ii) "Trojan horse" theory, and (iii) viral entry into the BMECs, transcytosis, and the release of virus into the brain parenchyma (Ruzek *et al.*, 2011).

Nonproteinous bacterial products and BBB translocation

Proteins from microbial pathogens are the dominant virulence factors mediating entrance to the CNS; however, various nonproteinous microbial components including lipopolysaccharide, LTA, glycolipids, and hyaluronic acid contribute to breakdown of the BBB. Lipooligosaccharide on the outer membrane is an important inflammatory agent in the CSF. Recent studies have demonstrated that lipooligosaccharide and lipopolysaccharide containing outer membrane vesicles provoke meningeal inflammation, increase concentration of leukocytes, and change permeability of the BBB (Cope *et al.*, 1990). Hyaluronic acid of *C. neoformans* capsule facilitates the transport via BBB (Jong *et al.*, 2007). Several hyaluronic acid receptors have been identified on various ECs; however, the only receptor on BMEC interacting with hyaluronic acid is CD44, the most common hyaluronic acid receptor in vertebrates. This interaction initiates the events of the entry at the BMEC membrane rafts (Jong *et al.*, 2008).

Exploitation of host's proteases by pathogens to degrade ECM of BBB

Plasmin- and plasminogen-mediated BBB translocation

Gram-positive and negative bacteria are able to express surface receptors for proteases that digest ECM and components of basal membrane. This is an important strategy of pathogens to cross various barriers. Serine protease plasmin degrades many blood plasma proteins, mostly fibrin clots. In serum, free plasmin is quickly inactivated by α_1-antiplasmin and α_2-antiplasmin (Mayer, 1990); however, cell surface-associated plasmin cannot be regulated by the serum inhibitor and degrades high–molecular weight glycoproteins such as fibronectin, laminin, and collagen IV which are essential for proper BBB function Fig. 3.

Most of the bacterial plasminogen receptors are extracellular metabolic enzymes (Pancholi *et al.*, 2003), which fall into two major categories: (1) filamentous protein structures that are morphologically similar to fibrin–fimbriae proteins and (2) nonfilamentous surface proteins, usually abundant proteins, with enzymatic activity and multiple-binding properties (Mayer, 1990). The nonfilamentous plasminogen receptors have relatively low affinity for plasminogen, which recognizes the lysine-binding sites of a receptor molecule (Lahteenmaki *et al.*, 1995). Fimbriae and flagella form a major group of plasminogen receptors in Gram-negative bacteria, whereas surface-bound enzyme molecules and M protein-related structures possess affinity to plasminogen in Gram-positive bacteria (Lahteenmaki *et al.*, 2001).

For the first time, binding of human plasmin to bacteria was reported for *Streptococcus* Group A. Over the next years, exploitation of host's plasmin and plasminogen for proteolysis of ECM, mediated by their surface proteins, was showed in many other bacteria like *Staphylococcus aureus*, *N. meningitidis*, *Neisseria gonorrhoeae*, *Yersinia pestis*, *B. burgdorferi*, and *Cronobacter sakazakii*. Binding of plasminogen to receptors of *B. burgdorferi*, *Borrelia hermsii*, *M. tuberculosis*, and *Streptococcus* Group A takes place via lysine residues (Coleman *et al.*, 1995). ErpP, ErpA, and ErpC proteins are the major plasminogen-binding proteins of *B. burgdorferi* (Brissette *et al.*, 2009).

It has been shown that plasminogen bound to the surface of *B. burgdorferi* can be activated and turn into plasmin by urokinase-type plasminogen activator (Hu *et al.*, 1995). Similarly, outer membrane protease (Cpa) of *C. sakazakii* causes uncontrolled plasmin activity by converting plasminogen to plasmin and inactivating the α2-antiplasmin (Franco *et al.*, 2011).

GlnA1, one of the few plasminogen receptors of *M. tuberculosis*, binds host's fibronectin to degrade ECM (Xolalpa *et al.*, 2007), while *C. albicans* binds both plasminogen and plasmin. Binding of *Candida* enolase to plasmin is also lysine-dependent and can be inhibited with arginine, aspartate, and glutamate (Jong *et al.*, 2003). Direct binding of plasmin and plasminogen in *Streptococcus* group A is mediated by three receptors: 1) plasminogen-binding group A streptococcal M-like protein, 2) α-enolase, and 3) glyceraldehyde-3-phosphate dehydrogenase (Lahteenmaki *et al.*, 2001; Lahteenmaki *et al.*, 2005). Surprisingly, *S. pyogenes* protein Prp does not interact with plasminogen and plasmin via lysine, however only via arginine and histidine residues (Sanderson-Smith *et al.*, 2007). GBS bind plasminogen only by the glyceraldehyde-3-phosphate dehydrogenase (Seifert *et al.*, 2003).

Metalloprotease-mediated BBB crossing

Matrix metalloproteinases/metalloproteases (MMPs) are zinc- or cobalt-dependent enzymes that play a crucial role in normal function and development of CNS. This large group includes collagenases, gelatinases, stromelysins, matrilysin, membrane-type metalloproteinases, and metalloelastases. MMPs differ in cellular sources and substrate specificity, but structural domains remain the same (Kieseier *et al.*, 1999). MMPs may alter inflammatory cytokine activity, cleave cell surface receptors, activate caspase-3, and regulate other MMP family members (Kawasaki *et al.*, 2008). Together with serine and cysteine proteases, they are able to degenerate and remodulate connective tissues. This damage leads to extravasation of blood-borne proteins, formation of brain edema, and neuronal damage. Pathogens exploit this extravasation to cross various barriers including BBB.

Basal level of MMP expression in the brain is low; however, during infections, basal level of MMP expression elevates markedly. MMPs are expressed by most of the resident CNS cells such as ECs, astrocytes, microglia, and neurons together with the infiltrating immune cells (Hummel *et al.*, 2001).

Infection of BMECs with neurotropic viruses has been connected with decrease and/or redistribution of TJ proteins (Luabeya *et al.*, 2000). MMP activity is highly increased in HIV-infected cells migrating into CNS. Human neuronal and glial cells infected with this virus have been shown to produce large amounts of MMP-2 (Chong *et al.*, 1998). During the WNV infection, it has been observed that inflammatory cytokines, such as TNF-α, macrophage migration inhibitory factor, and MMP-9 play an essential role in BBB disruption (Wang *et al.*, 2004;

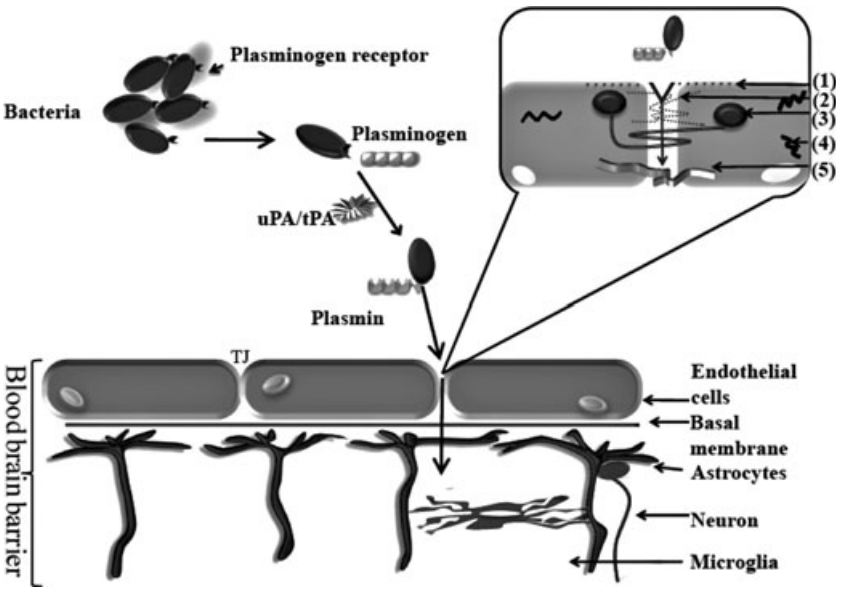

Fig. 3. Activation and role of plasmin(ogen) in the pathogen invasion. 1-laminin, 2-occludin, 3-zonula occludens, 4-actin, 5-collagen IV. Many bacteria express plasmin(ogen) receptors on their surface. Plasminogen is activated by the urokinase-type plasminogen activator/tissue plasminogen activator (uPA/tPA) to plasmin. Plasmin activates MMPs and degrades laminin and collagen IV. Degradation of these two proteins can alter the microvascular integrity and increases BBB permeability, this facilities transport of bacteria to the brain microenvironment.

Arjona *et al.*, 2007). It is likely that activation of MMP-9 in WNV-infected astrocytes is via MMP-3 (Verma *et al.*, 2010).

MMPs also play an important role in bacterial meningitis. In fact, MMP-8 and MMP-9, but not MMP-2 and MMP-3, are upregulated in CSF during the meningitis caused by *H. influenzae*, *N. meningitidis*, and *S. pneumoniae* (Leppert *et al.*, 2000). *Treponema denticola* (Gaibani *et al.*, 2010) and cell wall of *Streptococcus suis* strongly stimulate the production of MMP-9, whereas zinc metalloproteinase ZmpC of *S. pneumoniae* cleaves human MMP-9 into its active form (Oggioni *et al.*, 2003), which leads to the BBB disruption (Jobin *et al.*, 2006). MMP-8 is also associated with tissue destruction during *Streptococcus sanguinis*, *N. meningitidis*, and *Fusobacterium nuclearum* infections (Shin *et al.*, 2008; Schubert-Unkmeir *et al.*, 2010). Tissue destruction by *N. meningitidis* is a consequence of proteolysis of TJ protein occludin by MMP-8. Furthermore, MMP-8 activity also participates in BMEC detachment from the underlying matrix that arose during extended time of infection with *N. meningitidis* (Schubert-Unkmeir *et al.*, 2010). Meningitis caused by *S. pneumoniae* in the neonatal rats is associated with the higher expression of MMP-3, MMP-8, and MMP-9, whereas in rabbits, only MMP-2 and MMP-9 are found to be responsible for the impairment of BBB and blood–CSF barriers (Azeh *et al.*, 1998). *Mycobacterium tuberculosis* uses MMPs more effectively for the tissue and neural damage. Infected monocytes induce MMP-9 secretion from astrocytes, afforded by IL-1β and TNF-α (Harris *et al.*, 2007). The importance of MMP-9 in BBB disruption was proved elsewhere by diminishing the process of BBB disruption in MMP-9 knockout mice (Asahi *et al.*, 2001). *Borrelia burgdorferi* causes the release of MMP-1 and MMP-9 from human cells, while plasmin-coated *B. burgdorferi* stimulates pro-MMP-9. This triggers a cascade that leads to the degradation of basement membranes (Gebbia *et al.*, 2001). *Borrelia burgdorferi–Anaplasma phagocytophilum* coinfection of BMECs leads to increased reductions in transendothelial electrical resistance and elevated production of MMPs (MMP-1, MMP-3, MMP-7, MMP-8, and MMP-9) (Grab *et al.*, 2007). Together with other factors, such as cytokines and chemokines, this expression leads to the increase in vascular permeability and inflammatory responses. In fact, coinfection results in the higher production of MMPs than *B. burgdorferi* alone (Grab *et al.*, 2007). *Acanthamoeba* serine proteases have been demonstrated to disrupt human BMEC monolayers (Alsam *et al.*, 2005). Moreover, to the serine proteases, *Acanthamoeba* is able to use metalloproteinase activity (Sissons *et al.*, 2006). In general, expression of MMP-9 during the bacterial meningitis is 10- to 1000-fold higher than in the cases of viral meningitis (Kolb *et al.*, 1998).

Conclusion

Interactions between protein molecules from host and pathogens are crucial to trigger translocation processes. Indeed, it takes two to tango: both host receptors and pathogen ligands. Underlying molecular basis of BBB translocation by various pathogens has been revealed in the last decade, however, yet an array of protein–protein interactions between many of the neuroinvasive pathogens and BBB remained fully unexplored. Identification and molecular characterization of these pathogens and host factors mediating BBB penetration can open novel perspectives in the development of more specific drugs and vaccine strategies.

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Authors' contribution

E.B. and P.M. contributed equally to this work.

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