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MINIREVIEW

Pathogen translocation across the blood–brain barrier

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Abstract

Neurological manifestations caused by neuroinvading pathogens are typically attributed to penetration of the blood–brain barrier (BBB) and invasion of the central nervous system. However, the mechanisms used by many pathogens (such as *Borrelia*) to traverse the BBB are still unclear. Recent studies revealed that microbial translocation across the BBB must involve a repertoire of microbial–host interactions (receptor–ligand interactions). However, the array of interacting molecules responsible for the borrelial translocation is not yet clearly known. Pathogens bind several host molecules (plasminogen, glycosaminoglycans, factor H, etc.) that might mediate endothelial interactions *in vivo*. This review summarizes our current understanding of the pathogenic mechanisms involved in the translocation of the BBB by neuroinvasive pathogens.

Introduction

The blood–brain barrier (BBB) is a regulatory interface between the peripheral circulation and the central nervous system (CNS) (Kim, 2008). The neurological symptoms of some diseases are associated with the mode of traversal of this barrier and penetration into the brain by several pathogens (bacteria, fungi, parasites and viruses). Interestingly, some of the neuroinvasive pathogens, for example *Chlamydiophila pneumoniae* (MacIntyre *et al.*, 2002) and *Borrelia burgdorferi sensu lato* (Miklossy *et al.*, 2004; Batinac *et al.*, 2007), are reported to be associated with multiple sclerosis and Alzheimer's disease (AD), respectively. However, the relationship between these organisms and diseases remains unclear. Pathogens exploit several mechanisms that enable them to reach the CNS, such as the traversal of BBB or penetration through neurons by axonal flow. Many pathogens have the potential to infect the CNS, but it is unclear why only a relatively small number of pathogens account for most clinical cases with nervous disorders. A comprehensive understanding of BBB crossing mechanisms is pivotal for drug and vaccine development against neuroinvading pathogens.

The BBB

The BBB is a structural and functional barrier that regulates the passage of blood-borne substances and cells into the brain and thus maintains the homeostasis of the neural microenvironment that is crucial for normal neuronal activity and function (Abbott *et al.*, 2006). BBB is formed by brain microvascular endothelial cells (BMECs) that line the cerebral microvessels. The periendothelial structures of the BBB include pericytes (related to smooth muscle cells, surround the endothelium, reduce endothelial apoptosis and stabilize the endothelium), astrocytes (induce many BBB features and support the tissue of the CNS) and a basal membrane. The BMECs utilize unique features that distinguish them from the peripheral endothelial cells. Most prominent among these are as follows: (1) numerous intercellular 'tight junctions (TJs)' that possess high transendothelial electrical resistance and retard paracellular flux; (2) the absence of fenestrae and a reduced level of fluid-phase endocytosis; and (3) asymmetrically localized enzymes and carrier-mediated transport systems (Biegel *et al.*, 1995). The BMECs express several influx/efflux transporters that transport important nutrients such as glucose (GLUT-1

transporter), amino acids (LAT1 transporter) and nucleosides (e.g. ENT1 and CNT1) (de Boer *et al.*, 2003). Because of the negatively charged abluminal membrane, negatively charged molecules face obscurity while entering or exiting the endothelial cells. There are two families of multispecific anion transporters that mediate the influx and/or the efflux of these compounds: the organic anion transporter polypeptide family and the organic anion transporter (Sekine *et al.*, 2000). Transporters for organic cations OCNT1 (Tsuji, 2005) and monocarboxylic acids MCT1 and MCT2 (Gerhart *et al.*, 1989; Price *et al.*, 1998) have also been described on BMEC. Peptides and proteins such as insulin or transferrin are transported through the BBB by receptor-mediated transport (Davidson *et al.*, 1990; Moos *et al.*, 2007).

To protect the nervous system from xenobiotics, BMEC express several efflux transporters. The most important are members of the ATP-binding cassette (ABC) family, for example P-glycoprotein (ABCB1), multidrug resistance proteins (MRP1–6) and brain multidrug resistance protein (Deli *et al.*, 2005). Furthermore, metabolizing enzymes such as members of the cytochrome P450 family effectively restrict the entry of xenobiotics into the CNS (el-Bacha & Minn, 1999).

The TJs

Polarized epithelial cells carry out functions such as the transport of ions and nutrients, secretion of protein products and protection of the interior of the organism from invading microorganisms. Cell polarity is observed in the functionally distinct portions of the plasma membrane, known as the apical domain and the basolateral domain (Miyoshi & Takai, 2005). The apical domain contains anion channels, H^+/K^+-ATPase and transporters, whereas the lateral portion of the basolateral domain contains proteins involved in attachment to neighboring cells and cell–cell communication. The basal portion of the basolateral domain contains the binding sites for constituents of the basal lamina, and receptors for hormones and other signaling molecules that regulate the function of the cell (Fig. 1). The junctional complexes of the cerebral microvasculature include TJs (Wolburg & Lippoldt, 2002), adherens junctions (Schulze & Firth, 1993) and gap junctions (Nagasawa *et al.*, 2006).

TJs, which are localized to the apical end of the basolateral membrane, play a key role in establishing endothelial polarity. The presence of TJs leads to high transendothelial electrical resistance values of up to $2000\,\Omega\,cm^2$ as compared with $3–33\,\Omega\,cm^2$ in other peripheral tissues, and this results in lower paracellular permeability (Crone & Olesen, 1982). TJs also serve as fences between the apical and the basolateral domains of the plasma membranes in epithelial cells, preventing the diffusion of integral proteins and lipids from one to the other (Tsukita *et al.*, 2001).

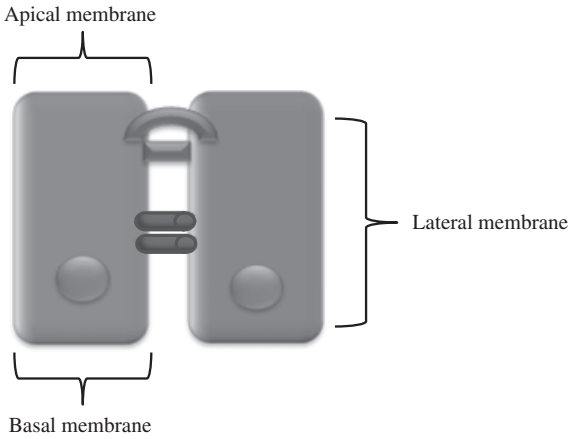

Fig. 1. Organization of the intercellular junctions. Cell attached to the adjacent cell via tight junctions (arc), adherens junctions (rectangle, provide strong mechanical attachment) and gap junctions (round-end rods, free intercellular channels that permit free passage of ions and molecules between the cells).

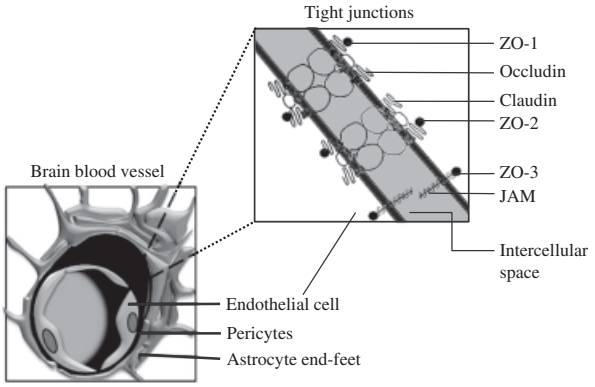

Fig. 2. The BBB and TJ.

An understanding the functional architecture of TJs has been achieved by identifying key components of adhesion systems. TJs of the cerebral microvasculate are composed of four integral membrane proteins – occludin (Furuse *et al.*, 1994), claudins (Furuse *et al.*, 1998), junctional adhesion molecules (Martin-Padura *et al.*, 1998) and the recently discovered endothelial cell-selective adhesion molecule (Nasdala *et al.*, 2002). These are linked through cytoplasmic proteins (e.g. ZO-1, -2, -3, cingulin) to the actin cytoskeleton (Wolburg & Lippoldt, 2002) (Fig. 2).

Traversal of BBB by pathogens

Several pathogens are able to cross the BBB and infect the CNS (Table 1). Pathogens may cross the BBB transcellulary, paracellulary and/or by means of infected phagocytes (the so-called Trojan horse mechanism) (Kim, 2006) (Fig. 3).

Table 1. Pathogens causing CNS infections in humans

Bacteria	*E. coli*, group B *Streptococci*, *Listeria monocytogenes*, *S. pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type B, *Citrobacter* spp., *Borrelia burgdorferi sensu lato*, *Treponema pallidum*, *Acinetobacter baumanni*, *Serratia marcescens*, *Pseudomonas putida*, *Enterococcus faecalis*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Meningococcus*, *Salmonella meningitis*, *Bacillus anthracis*, *Bacillus cereus*, *Francisella tularensis*, *Chryseobacterium meningosepticum*, *Kingella kingae*, *Rothia mucilaginosa* and *Mycobacterium tuberculosis*
Fungi	*Cryptococcus*, *Candida albicans*, *Aspergillus*, *Zygomycetes*, *Blastomyces*, *Histoplasma capsulatum*, *Cladophialophora bantiana*, *Coccidioides immitis*, *Pseudallescheria boydii*, *Arthrographis kalrae*, *Exophiala dermatitidis*, *Ramichloridium mackenzie* and *Ochroconis gallopava*
Parasites	*Plasmodium falciparum*, *Trypanosoma* spp., *Toxoplasma gondii*, *Teania solium*, *Naegleria fowleri*, *Acanthomoeba* and *Angiostrongylus cantonensis*
Viruses	HIV-1, herpes simplex virus, rhabdovirus (rabies), influenza virus, parainfluenza virus, reovirus, lymphocytic choriomeningitis virus, arbovirus, cytomegalovirus, flaviviruses (West Nile virus, Japanese encephalitis virus, tick-borne virus, St Luis encephalitis virus, Murray Valley encephalitis virus), mumps virus, parvovirus B 19, measles virus, T-cell leukemia virus, enterovirus, morbillivirus (Nipah and Hendra virus), bunyaviruses and togaviruses

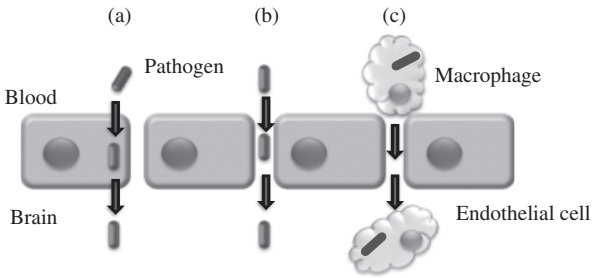

Fig. 3. Mechanisms of traversal of the BBB by pathogens. (a) Transcellular passage (without evidence of TJs disruption or detection of pathogens between the cells). (b) Paracellular passage (between cells with or without disruption of TJs). (c) Trojan horse mechanism (penetration within infected phagocytes).

Transcellular traversal of the BBB has been demonstrated for several bacterial pathogens, such as *Escherichia coli* (Kim, 2002), group B *Streptococcus* (Nizet *et al.*, 1997a, b), *Streptococcus pneumoniae* (Ring *et al.*, 1998), *Listeria monocytogenes* (Greiffenberg *et al.*, 1998), *Mycobacterium tuberculosis* (Jain *et al.*, 2006), fungal pathogens such as *Candida albicans* (*Jong et al.*, 2001) and *Cryptococcus neoformans* (Chang *et al.*, 2004), and is suggested for the West Nile virus (WNV) (Verma *et al.*, 2009). Paracellular penetration of the BBB has been suggested for the *Trypanosoma* sp. (Grab & Kennedy, 2008). In the Trojan horse mechanism, infected phagocytes carry the pathogen through the BBB. This mechanism has been suggested for *L. monocytogenes*, *M. tuberculosis* (Drevets *et al.*, 2004; Nguyen & Pieters, 2005) and HIV (Toborek *et al.*, 2005).

The pattern of borrelial crossing of the vascular endothelium (paracellular vs. transcellular) remains controversial. Comstock and Thomas (Comstock *et al.*, 1993) demonstrated that *B. burgdorferi* is able to translocate across the cytoplasm of human umbilical vein endothelial cell. However, not all studies have supported a transcellular route of crossing; Szczepanski *et al.* (1990) interpreted the presence of *B. burgdorferi* in the intercellular junctions between endothelial cells, as well as beneath the monolayers, as evidence that spirochetes actually pass between the cells.

Transversal mechanisms of the neuroinvasive bacteria, fungi, parasites, viruses and spirochetes are described below and the ligand–receptor interactions are summarized in Table 2.

Bacteria

Pathogenic bacteria have exploited various strategies to penetrate host cells. Ligand–receptor interactions are avoidable in the penetration of pathogens through the BBB (Table 2). Studies in humans and experimental animals point to a relationship between the level of bacteremia and the development of meningitis due to *E. coli* (Kim, 2002), group B *Streptococcus* (Ferrieri *et al.*, 1980) and *S. pneumoniae*. However, a high bacteremia level is necessary, but not sufficient, for BBB adhesion and traversal (Kim, 2006). Recent findings indicate that *E. coli* invades human BMEC through ligand–receptor interactions. Pivotal steps in the meningitis pathogenesis are microbial binding and invasion of BMEC. *Escherichia coli* determinants contributing to invasion have been identified – Ibe proteins and cytotoxic necrotizing factor 1 (CNF1) (Kim, 2006). CNF1 has been suggested to be internalized via receptor-mediated endocytosis upon binding to a cell surface of receptor 37-kDa laminin receptor precursor/67-kDa laminin receptor (Chung *et al.*, 2003). CNF1 mediates Ras homolog gene family, member A (RhoA), activation (Khan *et al.*, 2002).

Recent studies also indicated that other meningeal pathogens invade human BMEC via ligand–receptor interactions. For example, *S. pneumoniae* invades BMEC in part via an interaction between cell wall phosphorylcholine and the BMEC platelet activating factor receptor (Ring *et al.*, 1998). *Listeria monocytogenes* invasion of BMEC has been shown to

Table 2. Interaction of pathogen and host ligands used in binding, penetration and invasion of the BBB

Pathogen	Predicted way of BBB penetration	Ligand (pathogen)	Ligand (host)	References
E. coli	Transcellular	CNF1 (cytotoxic necrotizing factor 1)	37 LRP (laminin receptor precursor)	Chung *et al*. (2003)
			67 LRP	Khan *et al*. (2007)
		FimH	CD48	Prasadarao *et al*. (2003)
		OmpA	Gp96	Huang *et al*. (1995)
		IbeA	45-kDa protein	
S. pneumoniae	Transcellular	Phosphorylcholin	Platelet-activating factor receptor	Ring *et al*. (1998)
L. monocytogenes	Transcellular	Internalin B	gC1q-R (receptor for the globular	Greiffenberg *et al*. (1998)
	Trojan horse	Vip	head of the complement component	Braun *et al*. (1998)
	mechanism	ND	C1q) Met receptor tyrosine kinase	Shen *et al*. (2000)
			gp96	Cabanes *et al*. (2005)
			(glycoprotein 96)	Drevets *et al*. (2004)
			ND	
Neisseria meningitidis	Transcellular	Opc (outer membrane protein) Pili (Pil A and Pil B) LOS (lipooligosaccharide)	Fibronectin (anchoring to the integrin-$\alpha_5\beta_1$ receptor) CD46	Unkmeir *et al*. (2002)
			ND	Nassif *et al*. (1999), Johansson *et al*. (2003), Sokolova *et al*. (2004)
				Plant *et al*. (2006)
Group B *Streptococci*	Transcellular	Glycosyltransferase	ND	Doran *et al*. (2005)
		LTA (lipotechoic acid)	Laminin	Tenenbaum *et al*. (2007)
		Lmb	Fibrinogen	Tenenbaum *et al*. (2005)
		FbsA	ND	Maisey *et al*. (2007)
		Pili (PilA and PilB) iagA	ND	Doran *et al*. (2005)
Treponema pallidum	Paracellular	ND	ND	Thomas *et al*. (1988)
Haemophilus influenzae	ND	LOS	ND	Mustafa *et al*. (1989)
type B		phosphorylcholine	PAF receptor	Weiser *et al*. (1998)
Borrelia burgdorferi s. l.	Transcellular	ND	ND	Comstock *et al*. (1993)
	Paracellular	Vsp1	Proteoglycans	Szczepanski *et al*. (1990)
		OspA	Platelet integrins	Fuchs *et al*. (1994)
		70-kDa PBP	Glycosaminoglycans	Rupprecht *et al*. (2006)
			Glycosphingolipids	Hu *et al*. (1997)
			Plasmin(ogen) proteoglycans	
			Plasmin(ogen)	
Mycobacterium tuberculosis	Transcellular	Upregulation of genes Rv0980c, Rv0987c, Rv0989c, Rv1801	ND	Jain *et al*. (2006)
	Trojan horse mechanism		ND	Nguyen & Pieters (2005)
		ND		
Candida albicans	Transcellular	Enolase	Plasmin(ogen)	Jong *et al*. (2001, (2003), Lossinsky *et al*. (2006)
Cryptococcus neoformans	Transcellular	Hyaluronic acid	CD44	Chang *et al*. (2004)
		Phospholipase B	ND	Perfect & Casadevall (2002), Noverr *et al*. (2004), Olszewski *et al*. (2004), Shea *et al*. (2006), Jong *et al*. (2008)
		Urease	ND	
		Isc1	ND	
Histoplasma capsulatum	ND	Yps3p	TLR2	Aravalli *et al*. (2008)
West Nile virus	Transcellular	ND	ND	Verma *et al*. (2009)
Trypanosoma spp.	Paracellular	ND	ND	Grab & Kennedy (2008)
Plasmodium falciparum	ND	Pf-IRBCs or PfEMP1	Thrombospondin, CD36, ICAM1 (intercellular adhesion molecule) gC1qR, PECAM, VCAM1, ELAM, chondroitin sulfate A	Frigerio *et al*. (1998), Medana & Turner (2006), van der Heyde *et al*. (2006), Biswas *et al*. (2007), Tripathi *et al*. (2007)
Acanthomoeba	Paracellular	Mannose-binding protein	ND	Alsam *et al*. (2003)
Toxoplasma gondii	ND	SAG1	ND	Fischer *et al*. (1997)
HIV	Trojan horse mechanism	Tat protein	ND	Rappaport *et al*. (1999), Andras *et al*. (2003)
		ND	CD4 and CCR5	
	Transcellular	ND	ND	Kramer-Hammerle *et al*. (2005) Cavrois *et al*. (2008)

ND, not defined.

be mediated by internalin B (Greiffenberg *et al.*, 1998). *Neisseria meningitidis* invasion of human BMEC is mediated by a bacterial outer membrane protein, OspC, binding to fibronectin, thereby anchoring the bacteria to the integrin a5b1 receptor on the human BMEC surface (Unkmeir *et al.*, 2002). Further studies are needed to understand the contribution of these interactions to BMEC invasion and BBB traversal.

Previous studies revealed that internalized bacteria are found within membrane-bounded vacuoles of BMEC and transmigrate without multiplication and are protected from fusion with lysosomes (Kim, 2003, 2006). Electron microscopy studies have shown that *E. coli*, *M. tuberculosis* and group B *Streptococcus* invasion is associated with microvilli-like protrusions at the entry site on the surface of human BMEC (Nizet *et al.*, 1997a, b), suggesting a rearrangement of the host cell actin cytoskeleton. Actin cytoskeleton rearrangements are necessary for BMEC invasion by meningitis-causing bacteria, but the signaling mechanisms involved in actin differ among meningitis-causing bacterial species.

Citrobacter spp. are gram-negative bacteria and are associated with neonatal meningitis (Badger *et al.*, 1999). The unique feature of meningitis caused by *Citrobacter* spp. is their frequent association with brain abscess formation. The pathogenesis of *Citrobacter* spp. meningitis and brain abscess is not well characterized. *Citrobacter freundii* is able to invade and cross human BMECs *in vitro*. Invasion of BMECs by *C. freundii* was found to be dependent on microfilaments, microtubules, endosome acidification and *de novo* protein synthesis. In contrast to other meningitis-causing bacteria, *C. freundii* is able to multiply within human BMECs. This may be a mechanism whereby *C. freundii* traverses the BBB (Huang *et al.*, 2000).

Fungi

Several fungi have been shown to cause CNS infections in humans. In HIV-endemic areas *C. neoformans* (Gordon *et al.*, 2000) and *C. albicans* (Issel, 1971) are the most frequently isolated yeasts from patients with CNS involvement.

Cryptococcus neoformans is a common cause of culture-proven meningitis in areas where HIV-1 is endemic (Perfect & Casadevall, 2002). Brain invasion does not require the recruitment of host inflammatory cells (Chretien *et al.*, 2002; Chang *et al.*, 2004), which eliminates the possibility of the Trojan horse mechanism. Recent studies indicate that *C. neoformans* uses a transcellular mechanism of BMEC (Chang *et al.*, 2004) and requires protein kinase C-α activation (Jong *et al.*, 2008). The CPS1 gene is required for *C. neoformans* adherence to the surface protein CD44 of human brain microvascular endothelial cells (HBMECs) (Jong *et al.*, 2008).

Candida albicans is able to adhere, invade and transcytose across HBMEC without affecting the integrity of the monolayers (Jong *et al.*, 2001) by a poorly understood process. A *Candida* enolase interacting with the plasminogen system contributes to *C. albicans* invasion and traversal of human BMEC (Jong *et al.*, 2001).

Histoplasma capsulatum is a common cause of fungal infection, and although most infections are asymptomatic, it is capable of causing histoplasmosis in immunocompromised individuals (Aravalli *et al.*, 2008). *Histoplasma capsulatum* may cause meningitis in 5–25% of its victims who have AIDS. The interaction of *H. capsulatum* Yps3p with microglial cells leads to nuclear factor-κB activation via the Toll-like receptor 2 (TLR2) pathway (Aravalli *et al.*, 2008). A deeper understanding of the host–*Histoplasma* interaction is needed.

Parasites

Malaria seems to be a major public health problem in many parts of the tropical world. One of the important virulence mechanisms in *Plasmodium falciparum* is the ability of *P. falciparum* trophozoites and schizonts to sequester in the vasculature of diverse host organs (brain) (MacPherson *et al.*, 1985; Silamut *et al.*, 1999). *Plasmodium falciparum*-infected RBCs use the 32-kDa human protein gC1qR/HABP1/p32 as a receptor to bind to HBMECs (Biswas *et al.*, 2007).

The neurological manifestations of sleeping sickness in humans caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are attributed to the penetration of the CNS by trypanosomes, but how trypanosomes cross the human BBB remains unclear (Grab & Kennedy, 2008). The forms of trypanosomes found in the bloodstream efficiently cross HBMECs by a paracellular route (Grab *et al.*, 2004). In rodent models, the parasite can pass through the BBB across or between endothelial cells. Interferon-γ has been shown to play an important role in regulating trypanosome trafficking into the brain (Masocha *et al.*, 2007). A trypanosome apoptotic factor expressed by *T. brucei* that mediates apoptosis in mouse-brain and human-brain vascular endothelial cells (HBVECs) was identified and characterized (Stiles *et al.*, 2004).

Pathogenic *Acanthomoeba* is the common cause of keratitis and rare, but fatal granulomatous amoebic encephalitis. The mechanism that the pathogen uses to cross the BBB is unclear. Some studies revealed the ability of several genotypes of *Acanthomoeba* to bind human BMEC and cause cytotoxicity in BMEC (Alsam *et al.*, 2003).

Encephalitis is a serious complication of the infection with the obligate intracellular parasite *Toxoplasma gondii*. Additional studies are needed to elucidate the mechanism involved in toxoplasmosis of the CNS.

Viruses

The penetration of HIV into the CNS through neurons by axonal flow, which occurs with the herpes virus and the 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 the BBB is considered to play a crucial role in the pathogenesis of viral meningitidis in the late phase of AIDS. Although this model gained rapid favor, recent studies challenge this model by showing that the vast majority of virions transmitted in trans originates 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 the case of HIV infection, the viral protein Tat has been shown to induce an inflammatory process in brain endothelial cells, or endothelial cell apoptosis (Andras *et al.*, 2003; Kim & Langridge, 2004), and to be able to disrupt the intercellular TJs.

WNV-associated encephalitis is characterized by disruption of the BBB, enhanced infiltration of immune cells into the CNS, microglia 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 the paracellular pathway, in which case, an increase in WNV RNA at earlier time points would be expected, due to passive diffusion (Verma *et al.*, 2009). WNV does not induce the cytopathic effect and induces an expression of claudin-1 and upregulation of vascular cell adhesion molecule-1 and E-selectin (Verma *et al.*, 2008).

Spirochetes

Neurosyphilis and neuroborreliosis are prototypes for spirochete infection of the CNS, but their pathogenesis remains unclear.

Treponema pallidum can invade through the intercellular junction of aortic endothelial cells (Thomas *et al.*, 1988), which suggests the usage of a paracellular mechanism of penetration of the vascular endothelium, but it is unclear whether a similar mechanism is involved in *T. pallidum* penetration of the BBB.

Borrelia strains traverse human BMEC without obvious changes in the integrity of the host cells (Grab *et al.*, 2005). This translocation is facilitated by host proteases, which are involved in the plasminogen activation system and fibrinolysis (Coleman *et al.*, 1995, 1997, 2001; Coleman & Benach, 2000, 2003), like in other pathogens of low genomic capacity. The fibrinolytic system linked by an activation cascade may lead to focal and transient degradation of TJ proteins, allowing *B. burgdorferi* to invade the CNS.

Although the role of plasmin in infection is important, there are other host proteases that could also be used to enhance the translocation of the host barriers such as matrix metalloproteinases (MMPs) (Gebbia *et al.*, 2001).

Neuroborreliosis and the transversal mechanisms exploited by *Borrelia* are discussed below in detail.

Neuroborreliosis and neuroinvasive/transversal mechanisms

Lyme borreliosis (LB) is the most common tick-borne disease in the Northern hemisphere. Neuroborreliosis can arise at any time during the course of LB. For early neuroborreliosis, aseptic meningitis and an involvement of cranial and peripheral nerves are typical (Steere, 2001). Neuroborreliosis occurs in 15–25% of patients with localized erythema migrans. The most pronounced clinical symptom is pain, as a result of radiculoneuritis, headaches and facial palsy. During the course of neuroborreliosis, facial nerves are most frequently affected, resulting in unilateral or bilateral peripheral facial palsy (Nigrovic *et al.*, 2008).

Several bacteria express their own proteases, which digest extracellular matrices in order to invade tissues, but other bacteria, such as *B. burgdorferi*, appear to utilize the fibrinolytic system of the host to disseminate. *Borrelia burgdorferi* does not produce any collagenase, elastase, hyaluronidase or plasminogen activators (Klempner *et al.*, 1995). *Borrelia burgdorferi* is able to bind both human plasminogen and plasmin via various binding structures, mainly via OspA (Fuchs *et al.*, 1994) and a 70-kDa protein (Hu *et al.*, 1997). OspA expression is downregulated almost immediately after a blood meal by the tick vector and OspA is expressed minimally at all times of early infection in the mouse (Schwan *et al.*, 1995).

Plasminogen bonded on the bacterial surface can be converted in plasmin by host activators (Berge & Sjobring, 1993; Young *et al.*, 1998). Plasmin bonded to the surface of the bacterial cell is stabilized and protected against inactivation by α_1- and α_2-antiplasmin (Perides *et al.*, 1996). The protection of cell surface-bonded plasmin from physiological inhibitors may allow the spirochete to traverse normal tissue barriers, to colonize organs and to propagate pathological processes within the affected tissues. However, the fact that there is no difference in plasminogen binding between infectious and noninfectious strains of *B. burgdorferi* suggests that surface binding of plasmin or plasminogen is not the only determinant of virulence (Hu *et al.*, 1995).

Borrelia burgdorferi induces the expression and secretion of the urokinase-type plasminogen activator (uPA) and the expression of the uPA receptor (uPAR; CD87) by a variety of cell types, including monocytes (Coleman *et al.*, 2001;

Coleman & Benach, 2003). The uPAR synthesis can be induced through CD14 and TLR2 signaling, which establishes a new functional link between the PAS and the innate immune system (Coleman & Benach, 2003).

The fibrinolytic system can directly digest components of the extracellular matrix (Coleman *et al.*, 1999). It can also activate other proteases, including MMPs. *Borrelia burgdorferi* is capable of upregulating and activating human inflammatory cell MMPs (Gebbia *et al.*, 2001). Mononuclear cells and neutrophils may express many different MMPs that are often involved in host tissue destruction in various inflammatory diseases. These molecules could be used to penetrate various host barriers by an enhanced penetration across collagen I, laminin and collagen IV (Gebbia *et al.*, 2001), which are important constituents of the BBB.

The pathway for *B. burgdorferi* to activate or elicit an increase in the release of MMP-9 by host cells could either be direct or indirect (i.e. either by acting on cells via receptors) (Gebbia *et al.*, 2004) to increase their production of MMPs or by increasing the release of other mediators, such as cytokines, which could stimulate the release of MMPs (Zhao *et al.*, 2007). The upregulation of MMP-9 by *B. burgdorferi* could involve a CD14 signaling pathway. MMP-9 but not MMP-1 was specifically induced in monocytic cells through TLR2 by *B. burgdorferi* (Gebbia *et al.*, 2004).

Further investigations and challenges

This review summarizes and extends our understanding of how pathogens use different mechanisms to cross the BBB and invade the CNS. The neuroinvasive mechanisms used by many pathogens (such as *Borrelia*) are still unclear. Nevertheless, new pathogens are emerging with the potential of effective neuroinvasion, like the recent reports of neurofrancisellosis. The possible linkage between neuroinvasion by pathogens and disease conditions such as AD or MS has provided new and challenging research areas in neuroimmunology.

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