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SCIENCE DIRECT
International Journal for Parasitology 36 (2006) 607–614
www.elsevier.com/locate/ijpara

Invited Review

Microbial translocation of the blood–brain barrier

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Received 3 November 2005; received in revised form 19 January 2006; accepted 30 January 2006

Abstract

A major contributing factor to high mortality and morbidity associated with CNS infection is the incomplete understanding of the pathogenesis of this disease. Relatively small numbers of pathogens account for most cases of CNS infections in humans, but it is unclear how such pathogens cross the blood–brain barrier (BBB) and cause infections. The development of the in vitro BBB model using human brain microvascular endothelial cells has facilitated our understanding of the microbial translocation of the BBB, a key step for the acquisition of CNS infections. Recent studies have revealed that microbial translocation of the BBB involves host cell actin cytoskeletal rearrangements, most likely as the result of specific microbial–host interactions. A better understanding of microbial–host interactions that are involved in microbial translocation of the BBB should help in developing new strategies to prevent CNS infections. This review summarises our current understanding of the pathogenic mechanisms involved in translocation of the BBB by meningitis-causing bacteria, fungi and parasites.

Keywords: Central nervous system infection; Pathogenesis; Human brain microvascular endothelial cells; Blood–brain barrier; Signal transduction; Actin cytoskeleton rearrangement

1. Introduction

CNS infection continues to be an important cause of high mortality and morbidity throughout the world. For example, bacterial meningitis is recognised as one of the top 10 causes of infection-related death worldwide (Table 1) and approximately 30–50% of the survivors suffer neurological sequelae. A major contributing factor to this high mortality and morbidity is our incomplete understanding of the pathogenesis of this disease. As shown in Table 2, there are limited numbers of pathogens that are shown to be common in causing CNS infections in humans, but it is incompletely understood how those pathogens are able to cross the blood–brain barrier (BBB) and cause CNS infections. Pathogens may cross the BBB transcellularly, paracellularly and/or by means of infected phagocytes (so-called Trojan horse mechanism). Transcellular traversal of the BBB has been demonstrated for several bacterial pathogens, such as *Escherichia coli* (Kim, 2001, 2002, 2003), group B *Streptococcus* (Nizet et al., 1997), *Streptococcus pneumoniae* (Ring et al., 1998), *Listeria monocytogenes* (Greiffenberg et al., 1998), *Neisseria meningitidis* (Unkmeir et al., 2002), *Mycobacterium tuberculosis* (Jain et al., in press) and fungal pathogens such as *Candida albicans* (Jong et al., 2001) and *Cryptococcus neoformans* (Chang et al., 2004). Paracellular penetration of the BBB has been suggested for the protozoans *Trypanosoma* sp. (Grab et al., 2004). In the Trojan horse mechanism, infected phagocytes carry the pathogen through the BBB and this mechanism has been suggested for *L. monocytogenes* and *M. tuberculosis* (Drevets et al., 2004; Join-Lambert et al., 2005). Other routes of bacterial entry into the CNS include mechanical spread from a contiguous source of infection such as sinusitis and mastoiditis. For example, *S. pneumoniae* has been shown to enter the CNS through non-haematogenous route in experimental animals after intranasal infection and otitis media (Marra and Brigham, 2001). The availability of the in vitro BBB model enabled us to elucidate the mechanisms that are involved in microbial traversal of the BBB.

2. The blood–brain barrier

The BBB is a structural and functional barrier that is formed by brain microvascular endothelial cells (BMEC), astrocytes and pericytes. It regulates the passage of molecules into and out of the brain to maintain the neural microenvironment. BMEC

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0020-7519/$30.00
doi:10.1016/j.ijpara.2006.01.013

Table 1
Leading infectious causes of death worldwide, 2002

Cause	Rank	Estimated no. of deaths
Acute lower respiratory infections	1	3,884,000
HIV/AIDS	2	2,777,000
Diarrheal diseases	3	1,798,000
Tuberculosis	4	1,556,000
Malaria	5	1,272,000
Measles	6	611,000
Pertussis	7	294,000
Tetanus	8	214,000
Sexually transmitted diseases (excluding HIV)	9	180,000
Meningitis	10	173,000

The World Health Organisation (WHO), 2004.

possess distinct features such as tight junctions between them and low rates of pinocytosis (Rubin and Staddon, 1999). The BBB generally protects the brain from microbes and toxins circulating in the blood, and astrocytes and pericytes help maintain the barrier properties of BMEC. Recent studies, however, have shown that pathogens that cause CNS infection can cross the BBB as live organisms and cause CNS inflammation. At present, *E. coli*–BMEC interactions is the best characterised system concerning how CNS infection-causing pathogens cross the BBB (Kim, 2001, 2002, 2003; Xie et al., 2004; Kim et al., 2005a,b). The contributions of astrocytes and pericytes to microbial translocation of the BBB are shown to be minimal.

The in vitro BBB model is composed of human BMEC. Human BMEC were isolated from small fragments of brain specimens derived from children and adults (Stins et al., 1997, 2001). The resulting human BMEC were positive for factor VIII, carbonic anhydrase IV, Ulex Europaeus Agglutinin I, took up acetylated low-density lipoprotein (AcLDL) and expressed gamma-glutamyl transpeptidase, demonstrating their brain endothelial characteristics. Human BMEC were purified by fluorescence activated cell sorting (FACS) using fluorescently labeled DiI-AcLDL and found to be >99% pure after identifying non-endothelial cell types reacting to markers for astrocytes (glial fibrillary acidic protein), oligoglia (galactocerebroside-C) pericyte (smooth muscle actin), epithelial cell (cytokeratin) and microglia (macrophage antigens). In addition, cell morphology was examined.

Importantly, upon cultivation on collagen-coated Transwell inserts these human BMEC exhibited morphologic and functional properties of tight junction formation as well as a polar monolayer. These were confirmed by the demonstration of: (i) tight junction proteins (such as claudin 5 and ZO-1); (ii) adherens junction proteins (such as VE-cadherin and β-catenin); (iii) their spatial separation of these protein groups; (iv) limited transendothelial permeability to inulin (4 kDa) and dextran (70 kDa); and (v) development of high transendothelial electrical resistance (Stins et al., 2001; Kim et al., 2004; Ruffer et al., 2004).

Table 2
Pathogens commonly causing central nervous system infections in humans

Bacteria: *Escherichia coli*, group *B Streptococcus*, *Listeria monocytogenes*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type b, *Mycobacterium tuberculosis*, *Treponema pallidum*, *Borrelia burgdorferi*

Fungi: *Cryptococcus*, *Candida*, *Aspergillus*, *Zycomycetes*

Parasites: *Plasmodium falciparum*, *Trypanosoma* spp., *Toxoplasma gondii*, *Taenia solium*, *Naegleria*, *Acanthamoeba*

3. Microbial translocation of the blood–brain barrier

3.1. The use of in vitro models

We have established both in vitro and in vivo models of the BBB using human BMEC and experimental haematogenous meningitis in neonatal rats, respectively. Using both models, we have shown that *E. coli* invasion of human BMEC is a prerequisite for *E. coli* traversal of the BBB in vivo (Huang et al., 1995; Wang et al., 1999; Hoffman et al., 2000; Wang and Kim, 2002; Khan et al., 2002). Pathogenic microbes have exploited various strategies to penetrate host cells. Microbial internalisation into non-professional phagocytes such as epithelial and endothelial cells is shown to occur mainly via two different mechanisms involving the host cell actin cytoskeleton rearrangements. These are a zipper mechanism where cell protrusions are formed in contact with the pathogens and a trigger mechanism where a membrane ruffles around the pathogens (Knodler et al., 2001; Cossart and Sansonetti, 2004).

Studies have revealed that meningitis-causing bacteria such as *E. coli* and group B *Streptococcus* internalise in human BMEC via ligand–receptor interactions and invasion of human BMEC by meningitis-causing bacteria requires BMEC actin cytoskeleton rearrangements (Nizet et al., 1997; Nemani et al., 1999a,b). The mechanisms that are involved in human BMEC actin cytoskeleton rearrangements and invasion are, however, shown to differ between meningitis-causing bacteria (Kim, 2001, 2002, 2003).

Once meningitis-causing bacteria are inside human BMEC, they reside inside membrane-bound vacuoles (Nizet et al., 1997; Nemani et al., 1999a,b; Kim, 2003). Some bacteria modulate intracellular trafficking to avoid lysosomal fusion and avoid killing by lysosomal enzymes, which is an important attribute of a variety of organisms including meningitis-causing *E. coli* K1 (Kim et al., 2003).

3.2. Bacteria

Several bacterial species have been shown to be common causes of CNS infections (Table 2). How these bacteria cross the BBB and cause meningitis is incompletely understood. Recent studies have shown that successful crossing of the BBB by circulating *E. coli* requires: (i) a high degree of bacteremia; (ii) *E. coli* binding to and (iii) invading BMEC; (iv) rearrangements of BMEC actin cytoskeleton; and (v) traversal of the BBB as live bacteria (Kim, 2001, 2002, 2003).

Several studies in humans and experimental animals point to a relationship between the magnitude of bacteremia and the development of meningitis due to *E. coli*, group B