Both sides of the story How bacteria transfer through the blood–brain barrier?

Las dos caras de la historia ¿Cómo se transfieren las bacterias a través de la barrera hematoencefálica?

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ABSTRACT

Infections of central nervous system (CNS) still represent an important cause of mortality although globally abundance of antibiotics usage. Sepsis, bacterial invasion and microbial transversal of the Blood–Brain Barrier (BBB) are required to infect CNS. The latest research showed up that bacterial translocation through the BBB includes cytoskeleton rearrangements. Three different mechanism were described: paracellular, transcellular and Trojan-horse mechanism (in phagocytes). The consequences may be dramatic – disruptions of structure and loss of functionality of the BBB causing increased permeability, inflammatory and encephalopathy. Further experimental research should lead us to gain complete understanding of the host-bacteria interaction within microbial transversal of the BBB.

Keywords: blood–brain barrier, bacterial pathogens, infections, cell junction.

RESUMEN

Las infecciones del sistema nervioso central (SNC) siguen representando una importante causa de mortalidad a pesar de la abundancia mundial del uso de antibióticos. La sepsis, la invasión bacteriana y el traspaso microbiano de la barrera hematoencefálica (BBB) son necesarios para infectar el SNC. Las últimas investigaciones han demostrado que la translocación bacteriana a través de la BBB incluye reordenamientos del citoesqueleto. Se han descrito tres mecanismos diferentes: paracelular, transcelular y de caballo de Troya (en los fagocitos). Las consecuencias pueden ser dramáticas: alteraciones de la estructura y pérdida de funcionalidad de la BBB que provocan un aumento de la permeabilidad, inflamación y encefalopatía. Las nuevas investigaciones experimentales deberían llevarnos a comprender completamente la interacción huésped-bacteria en la transversalidad microbiana de la BBB.

Palabras clave: barrera hematoencefálica, patógenos bacterianos, infecciones, unión celular.

1 THE BRAIN-BLOOD BARRIER

The Blood–Brain Barrier (BBB) is created by the nervous and circulatory systems. It is a structural and functional barrier which performs extremely responsible functions. The BBB regulates molecules' inflow and outflow of the brain (maintaining stable environment) and protects neurons against toxins and pathogens. The vessels inside the brain consist of endothelium and wide range of junctions between cells (especially tight junction). The pinocytosis activity of endothelial cells is considered to be low. Astrocytes and pericytes are located on the outer site of a vessel. They prevent intercellular junction from leak (**Figure 1.**). This is not all. The structure of the BBB regulates transport of hydrophilic hormones and glucose to the brain. However, on the gases and hydrophobic hormones' transport the limitations are not placed [1].

Table 1. Types of cell junctions with details about structure and function.

Type of cell junctions	Structure and function	Literature
Tight Junction (TJ)	<ul style="list-style-type: none"> - fine and tight junctions between cells' membranes - occludins and claudins – homodimeric transmembrane proteins; they brings membranes of two cell closer - ZO-1/2 – proteins binding occludins and claudins with actin cytoskeleton 	[2, 3]
Adherens Junction (AJ)	<ul style="list-style-type: none"> - they provide fine intercellular adhesion, although flow of liquid through AJ is not limited - VE-cadherin – homodimeric proteins - α/β-catenin – responsables for binding cadherins with actin cytoskeleton - catenin p120 – regulates cadherin stability and maintains VE-cadherins surface expression 	[4]
Hemidesmosome	<ul style="list-style-type: none"> - provides attachment between the cell and collagenous basement membrane - integrine $\alpha_6\beta_4$ – laminin-332 receptor; binds cell's surface with basement membranes' protein elements - plectin – intermediates in binding transmembrane hemidesmosome's elements with cytoskeleton 	[5]

Figure 1. The Blood–Brain Barrier formed by: endothelial cells, pericytes, and astrocytes.

2 MICROBIAL TRANSVERSAL OF THE BBR MECHANISMS

Three different mechanisms of microbial transfer through the BBB were described: paracellular, transcellular and Trojan-horse mechanism (in phagocytes) [6]. For success of paracellular and transcellular mechanisms the most important issue is survival of bacteria in the host's blood [7]. After sepsis inducing and achievement of threshold bacterial density, pathogens get able to attach inside a brain vessel (because low intensity of this process). In contrast, in Trojan-horse mechanism it is possible to transport bacteria from distal tissues (e.g. small intestine) without sepsis inducing [8].

Table 2. Pathogenic bacteria mechanisms of survival inside the host blood.

Species	Inside blood survival mechanisms	Literature
<i>Porphyromonas gingivalis</i>	- gingipains – unspecific proteases which degrades complement system elements and extracellular matrix proteins of host's tissues	[9]
<i>Streptococcus pyogenes</i>	- M protein – block antibodies (binding Fc regions)	[10]
<i>Yersinia enterocolitica</i>	- YadA – unables the complement system to function properly - effectors secreted by T4SS – prevent actin cytoskeleton from rearrangement causing fagocytosis disruptions	[11, 12]
<i>Klebsiella pneumoniae</i>	- polysaccharide layer producing	[13]
<i>Streptococcus pneumoniae</i>	- TatD – DNase enabling NETs degradation (DNA nets connecting with bactericidal activity: LL37, myeloperoxidase, neutrophil elastase)	[14]

2.1 TRANSCELLULAR TRANSVERSAL

Bacteria transverse the BBB indirectly through the endothelial cell. Because of low pinocytosis activity, microbes have to induce it by activating particular receptors. Two types of inducible bacterial internalization may be distinguished: (i) “zipper” – a pathogen adheres to the cell and is slowly surrounded by membrane ruffles of the endothelial cell after particular receptors’ activation [15]; (ii) “trigger” – effectors, which cause endothelial cell’s cytoskeleton rearrangements, are transported indirectly to the cytoplasm through type III secretion system [16]. After successful internalization bacteria has to survive inside the phagosome. The brain-blood barrier is transfer by the phagosome. Endothelial cells are not susceptible to phagocytosis and do not possess any complicated lysosome system. However, the system of NLR proteins (NOD-like receptors) still can be activated. NOD-like receptors play role in immune response against intracellular pathogens [17]. After phagocyte transversion through the BBB, the exocytosis and membrane local disruption may occur [6].

2.2 PARACELLULAR TRANSVERSAL

In this case, bacteria induce disruption in intercellular junctions between endothelial cells. Proteases secreted by pathogens as well as boosted immune response activation because of strongly reactive antigens are thought to bring this effect. Some species e.g. *Neisseria meningitidis* activate secretion of host cell’ extracellular matrix proteases (MMP-8) [1], what includes dramatic damages of the neural tissue because of leak emergence. An extreme manifestation is complete degradation of the BBB. Bacterial cytolysins lead to disintegration of endothelial cells. The immune system also makes its contribution to pathological aggravation. Proinflammatory cytokines (e.g. TNF- α i IL-17) undermine stability of intercellular junctions (especially TJs) and increase leak through the BBB [18]. All this processes result in proceeding barrier degradation because of a positive feedback mechanism. Within brain vessels activation of the immune system makes the BBB more susceptible to leak. Bacterial replication form a vicious circle and activate secretion of proinflammatory cytokines in further steps of infection [6].

2.3 TROJAN-HORSE MECHANISM

This mechanism is characteristic for bacteria which are able to infect particular type of phagocytes, e.g. macrophages. Pathogens transverse the BBB inside phagocyte which prevent form the immune system activation and provide suitable environment to replicate [19, 20]. Survival in blood is not required. The bacteria may be engulfed by (for instance) a macrophage in different tissues also in regions of primary

infection. IL-1 β is one of the cytokines, which induce extravasation (leaving a vessel by a cell of the immune system) [6].

Figure 2. Three different mechanism transversal the Brain-Blood Barrier were described (sequentially): paracellular, transcellular and Trojan-horse mechanism (in phagocytes).

3 EXAMPLES OF BACTERIA ABLE TO TRANSFER THROUGH THE BBB

3.1 *STREPTOCOCCUS PNEUMONIAE*

To the present day *Streptococcus pneumoniae* is the main etiological factor in meningitis at the world. The first stage of human infection includes colonization of the nasopharynx. Transmission of the pathogen occurred person to person – via droplets (an airborne pathogen). Human can carry the bacteria without demonstrable symptoms [21]. About 30 per cent of infected persons are estimated to be unaware carriers. From nasopharynx mucous membranes *S. pneumoniae* can be relocated to lungs, tympanum and bloodstream. A well-developed polysaccharide layer provide protection for bacteria inside blood [22]. Some modifications of a polysaccharide layer occur at the stage of adhesion to the epithelial cells (it pertains to pathogens with a thin or without bacterial capsule). To thick polysaccharide layer enables bacteria to adhere to the surface, because interaction between adhesins and host cell receptors cannot occur. Surface neuraminidase (NanA) and RrgA adhesin belongs to bacterial proteins, which bind to endothelial cells. *S. pneumoniae* produces cytolytic toxin (pneumolysin), which is released as a result of bacteria lysis. Pneumolysin causes indirectly damage of the Brain-Blood Barrier (because of endothelial cell decomposition) [23–25].

3.2 *MYCOBACTERIUM TUBERCULOSIS*

Extrapulmonary infections of *M. tuberculosis* pertain especially to patients with immunodeficiency – infected with HIV, undernourished and after transplantation. In developing countries children are also susceptible to *M. tuberculosis* infections [26, 27]. This pathogen is highly adapted to persistence inside phagocytes. These cells are thought to be excellent niche inside infected lungs. Bacteria can be transferred inside phagocytes to other host tissues including bones and nervous tissue. The

development of infection is relatively slow, what enables microbes to achieve threshold bacterial density in blood necessary to cause sepsis [28]. However, *M. tuberculosis* transverse the BBB using Trojan-horse mechanism, so high bacterial density in blood is not essential [6].

3.3 *NEISSERIA MENINGITIDIS*

Infections of this coccus manifest in life-threatening meningitis. Five main pathogenic serotypes were identified by polysaccharide layer's chemical composition (called: A, B, C, Y and W135). Vaccines protecting against *N. meningitidis* were based on bacterial capsular antigens. However, a situation is more complicated in case of a serotype B. Surface polysaccharides of *N. meningitidis* serotype B resemble some nervous tissue antigens [29, 30]. The first stage of bacterial invasion of the central nervous system includes tight adhesion to the surface of endothelial cells. A few proteins and structures (type IV pilus, NadA adhesin, Opa and Opc proteins) are responsible for this process. Successful adhesion induce dramatic cytoskeletal rearrangements and decrease in intercellular junction stability [29]. Moreover, *N. meningitidis* activate host cells to secrete unspecified proteases, which start to degrade extracellular matrix elements including adhesins and basement membrane [6],

3.4 *ESCHERISCHIA COLI* K1

Tight adhesion of bacteria to the endothelium is crucial factor the successful invasion to nervous tissue. The main virulence factors at this stage is two elements: type 1 fimbriae and surface protein called OmpA. Fim H (terminal adhesin of the fimbriae type 1) interacts with CD48. OmpA protein has affinity with GlcNAc glycoproteins on the endothelium (e.g. gp96) [31, 32]. Surprisingly, this fact may be used in clinical practice in foreseeable future. Bacterial invasion on Central Nervous System can be blocked in a result of binding oligomers GlcNAc- β 1 and 4-GlcNAc, which are soluble in blood. They interact with OmpA proteins, unable bacteria to adhere to the endothelium and further infection development is impossible. CNF1 and IbeA are additional proteins which play key role in second stage of the brain invasion. They activate receptors and signaling pathways, what leads to cytoskeleton rearrangement (**Figure 3.**) [6].

Figure 3. Signalling mechanisms involved in actin cytoskeleton rearrangements mediated by Escherichia coli K1.

3.5 LISTERIA MONOCYTOGENES

L. monocytogenes Gram-positive pathogen causing gastroenteritis – listeriosis. Patients after chemotherapy treatment and these diagnosed with AIDS are extremely susceptible to get *L. monocytogenes* infection. This pathogen is highly dangerous for pregnant women, because infection may increase risk of miscarriage. *L. monocytogenes* similarly to pathogens mentioned above is also able to cause sepsis and brain infections. The most important molecular hallmark of listeriosis is persistence and replication inside phagocytes and the other host cells. At least 3 hydrolases are necessary for bacteria to leave the early phagosome: listeriolysin O (LLO), cholesterol-dependent cytolysin (CDC) and phospholipase C (PLC) [33, 34]. *In vitro* in stressing conditions *L. monocytogenes* produces intensively membrane vesicles (MVs) containing mainly LLO. Many other compounds were detected inside MVs, e.g. internalin B (InlB), catalase, p60 antigen, Lmo 1028 protein. Moreover, vesicles are able to transverse the Brain-Blood Barrier in regions where intercellular junctions are not leaktight [35]. *L. Monocytogenes* is thought to use three different mechanisms of bacterial translocation through the BBB: (I) paracellular, (II) Trojan-horse mechanism and (III) retrograde transfer inside cranial nerves axons (mainly vagus nerve) [6, 36, 37].

4 CONCLUSIONS

Microorganisms infecting the Central Nervous System cause serious damages in the brain structure. Effect of this may be more killing than the bacterial infection. Our knowledge about structure and physiology of vessels inside the brain and the brain-blood barrier is not complete. As a result progression of the preventing form infectious diseases of the central nervous system. *In vivo* (on model animals) and *in vitro* research leads us to partial understanding of interaction between pathogen and their host. To develop effective treatment, inhibitors of infection and fast diagnosis tools detailed description of pathogenesis mechanisms is necessary.

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