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Review

How Does *Streptococcus pneumoniae* Invade the Brain?

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Streptococcus pneumoniae **(the pneumococcus) is the major cause of bacterial meningitis. The mechanisms by which pneumococci from the bloodstream penetrate the blood–brain barrier to reach the brain are not fully understood. Receptor-mediated adhesion of the bacteria to the brain endothelium is considered a key event leading to meningitis development. The aim of this review is to discuss recent advances and perspectives related to the interactions of *S. pneumoniae* with the blood–brain barrier during the events leading to meningitis. Altogether, the available data suggest that, by precisely defining the pathways and ligands by which *S. pneumoniae* adheres to specific receptors, it may be possible to interfere with the respective mechanisms and develop strategies to prevent or even cure pneumococcal meningitis.**

Trends

Streptococcus pneumoniae, the main causative agent of bacterial meningitis, penetrates the blood–brain barrier by binding to PECAM-1 and pIgR expressed by brain vascular endothelial cells.

Overall, recent findings suggest that PECAM-1 and pIgR, together with platelet-activating factor receptor (PAFR), another receptor thought to be more involved in signaling rather than physical binding, might cooperate during adhesion of pneumococci to brain endothelial cells.

The passage of *S. pneumoniae* across the blood–brain barrier is a crucial step for meningitis development. The knowledge of how *S. pneumoniae* penetrates the blood–brain barrier can be used to develop strategies aimed at interfering with the respective pathways in order to prevent the entry of the pathogen into the brain.

Streptococcus pneumoniae, the Blood–Brain Barrier, and Pneumococcal Meningitis

The Gram-positive bacterium *Streptococcus pneumoniae* (the pneumococcus) is an important commensal resident of the human nasopharynx. Although carriage is usually asymptomatic, *S. pneumoniae* can become invasive and spread from the upper respiratory tract to other organs, leading to serious diseases such as pneumonia, sepsis, or meningitis [1,2]. *S. pneumoniae* is the major etiological cause of bacterial meningitis, responsible for two-thirds of meningitis cases in Europe and in the USA [3–5].

Bacterial meningitis is a disease with high morbidity and mortality worldwide despite the implementation of several vaccination programs and antimicrobial agents [3–6]. A major route for bacteria to reach the meninges is through the bloodstream [7]. Having reached the blood vessels in the brain, bacteria present in the bloodstream have to cross the blood–brain barrier to enter the brain and cause infection. This view is supported by recent immunofluorescent analyses combined with high-resolution confocal microscopy, where blood-borne *S. pneumoniae* was clearly shown to adhere to the brain vascular endothelium prior to invasion of the brain [8].

The blood–brain barrier is a specialized vasculature system that separates the brain from circulating blood and has critical functions in both protection and nutrient supply of the brain [9–11]. Endothelial cells form the layer that lines the interior surface of the blood vessels [12,13]. Pathogens can invade the brain only after crossing the endothelial cell layer of the blood–brain barrier and, therefore, they must develop strategies to pass this barrier. The inflammatory features and clinical complications following bacterial meningitis normally observed in humans can be reproduced using animal models. Notably, most *in vivo* models that examine the pathophysiology of bacterial meningitis involve the direct injection of pneumococci into the brain of mice or rats [14–18]. Administration of bacteria directly into the brain bypasses the need for blood–brain barrier translocation. In order to study the interaction of blood-borne

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S. pneumoniae with the brain vascular endothelium *in vivo*, prior to the onset of meningitis, a so-called bacteremia-derived meningitis model was established in recent years [8,19]. This model, where bacteria are injected into the bloodstream instead of the brain, has allowed the visualization of successive steps from bacteremia to brain invasion. The aim of the present review is to discuss recent advances in our understanding how blood-borne *S. pneumoniae* interacts with the blood–brain barrier endothelium during the events leading up to meningitis, and how to use such knowledge to develop new therapeutic strategies to fight the disease.

Receptor-Mediated Transcytosis

How bacterial pathogens cross the blood–brain barrier is still subject of debate. Several possible mechanisms have been implicated in this process, such as: (i) destruction of the endothelial cell layers in case of, for example, *Neisseria meningitidis* [20]; (ii) traversal of the blood–brain barrier in between the endothelial cells by disruption of tight junctions in case of group A streptococci [21]; and (iii) receptor-mediated transcytosis across endothelial and epithelial cell layers in case of *S. pneumoniae* [22,23]. The view that pneumococci pass the blood–brain barrier via transcytosis rather than pericellularly is supported by experiments, showing a continuous and homogeneous staining of VE-Cadherin in the brain of infected mice [8]. This implies that blood-borne *S. pneumoniae* does not cause a disruption of the endothelial tight junctions. In this context, it should be noted that bacterial pathogens such as pneumococci have the capability to bind to certain receptors on the plasma membrane of epithelial and endothelial cells, and this receptor-mediated binding facilitates bacterial invasion into and translocation over human cell layers. The passage of bacterial pathogens across such layers is a fundamental step for development of invasive diseases and it is necessary for *S. pneumoniae* to cross the blood–brain barrier to develop bacterial meningitis [22,23].

The Platelet-Activating Factor Receptor (PAFR)

The first reported receptor implicated in adhesion to, invasion of, and also transcytosis through endothelial cells is the PAFR. PAFR is a G-protein-coupled receptor with seven transmembrane domains and its natural ligand is the platelet-activating factor (PAF). PAF is a mediator in diverse pathologic processes, such as allergy, asthma, septic shock, arterial thrombosis, and inflammation [24–26]. Binding of PAF to the PAFR stimulates numerous signal transduction pathways including phospholipase C, D, A2, mitogen-activated protein kinases (MAPKs), and the phosphatidylinositol-calcium second messenger system [25]. PAFR has been proposed to bind *S. pneumoniae*, thereby facilitating adhesion to, uptake by, and transcytosis through endothelial cells leading to invasive disease [23,27,28]. However, while *in vitro* and *in vivo* studies indicate that PAFR is involved in the development of invasive pneumococcal disease (IPD), there is so far no unequivocal published evidence that a direct binding between *S. pneumoniae* and PAFR occurs [24]. A recent study suggests that PAFR plays a role in pneumococcal adhesion to endothelial cells, even though pneumococci do not directly bind to PAFR [29]. After immunofluorescent microscopy analysis, colocalization of PAFR and *S. pneumoniae* was not observed *in vivo* in mouse brain tissue nor in endothelial cells *in vitro*. Nevertheless, upon blockade of PAFR, adhesion of pneumococci to human endothelial cells was significantly reduced, indicating that PAFR most likely has an indirect role in IPD [29]. When the PAFR was genetically deleted or chemically inhibited, *S. pneumoniae* was still able to adhere to human cells *in vitro*, and to cause invasive disease *in vivo* [23,27].

The Laminin Receptor (LR)

The LR is an important molecule involved both in cell adhesion to the basement membrane and in signal transduction following this binding event. The LR on endothelial cells was found to interact with neurotropic viruses, including Sindbis virus [30], Dengue virus [31], adeno-associated virus [32], tick-borne encephalitis virus, and Venezuelan equine encephalitis virus [33]. LR was identified to be a common receptor for both *S. pneumoniae* and *N. meningitidis* on