The *Streptococcus suis* factor H-binding protein: A key to unlocking the blood-brain barrier and access the central nervous system?

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*Streptococcus suis* is one of the most important porcine bacterial pathogens, responsible for sudden death and meningitis, alongside a variety of other pathologies. Moreover, it is a zoonotic agent causing septic shock and meningitis in humans, being among the most important causes of adult bacterial meningitis in South-East Asia and responsible for two human outbreaks in China. Of the 35 described serotypes, serotype 2 is the most important worldwide with regards to both porcine and human cases of infection. However, serotype 2 strains are genotypically and phenotypically heterogeneous, with greatly varying virulence levels, fact that has complexified research on this pathogen.

The ability of *S. suis* to cause a variety of pathologies is the result of its ability to adapt to several hosts and environments. Being a natural inhabitant of pigs, *S. suis* colonizes the nasal and oral cavities, the tonsils, and the upper respiratory tract. Breaching of the mucosa is a critical step that allows the bacterium to reach the bloodstream where, following replication, bacteremia will lead to the development of systemic pathologies characteristic of this pathogen. In the case where the host survives the systemic infection, bacteria may reach the central nervous system (CNS) following entry via either the blood-brain barrier (BBB) or blood-cerebrospinal fluid barrier (BCSFB) to cause meningitis. However, entry of *S. suis* into the CNS remains largely misunderstood. While some studies have proposed the BCSFB as the main site of entry, the BBB represents a much more important surface area, thus allowing greater opportunities for the pathogen to traverse. Indeed, various studies have demonstrated that *S. suis* is capable of interacting with the brain microvascular endothelial cells (BMECs) constituting the BBB.

The unique pathogenesis of this bacterium is the result of its multitude of virulence factors, many of which are considered putative, with often misunderstood and controversial roles. Of these, the factor H-binding protein (Fhb) was first described as being involved in the recruitment of factor H to the *S. suis* surface and degradation of C3b into iC3b to reduce opsonophagocytosis, while also increasing adherence to and invasion of host cells. Moreover, this Fhb was also demonstrated to be a streptococcal adhesion P (SadP), which recognizes the galactosyl-a1–4 galactose moiety of the globotriaosylceramide (Gb3) cell receptor. However, Gb3 is only expressed by certain cell types, such as endothelial cells and, more particularly, BMECs. Interestingly, the *S. suis* Fhb, alongside its ability to bind factor H, was recently suggested to also contribute to the development of meningitis, though the mechanisms involved remain unknown. Moreover, while the interactions between bacteria and host cell surface carbohydrates have been suggested as critical initial steps for the development of disease, the role of Fhb-Gb3 interactions on the development of *S. suis* meningitis remain unknown.

In this issue of *Virulence*, Kong et al. investigated the role of the *S. suis* Fhb in the development of meningitis and the involved mechanisms. Using the human hCMEC/D3 BMEC cell line, the authors created a monolayer model of the human BBB which they challenged with an isolate from the highly virulent clonal strain responsible for the 2005 human outbreak in China, demonstrating that *S. suis* crosses this monolayer via the paracellular route only. This result was further confirmed by transmission electron microscopy and laser scanning fluorescence microscopy, where bacteria were observed at the cell margins whose tight junctions appeared...
disrupted or opened. While previous studies demonstrated a capacity of *S. suis* to invade porcine BMECs, only adhesion to, but not invasion of, human BMECs was reported. Through the use of a Fhb-deficient mutant and complemented strain, Kong *et al.* demonstrated that the Fhb is in part responsible for the capacity of *S. suis* to traverse the monolayer. By blocking Fhb using anti-Fhb IgGs, the authors confirmed this result whereby monolayer traversal was reduced. However, the lack of traversal abrogation suggests implication of additional bacterial factors in this event.

To further dissect the mechanisms involved in Fhb-dependent traversal of *S. suis* across the hCMEC/D3 monolayer, Kong *et al.* evaluated the role of Fhb-Gb3 binding in the adherence of *S. suis* to the monolayer by inhibiting the synthesis of Gb3 through the use of D-threo-1-phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP). This experiment was based on the previously reported binding of Fhb to the glycolipid receptor Gb3 via its N-terminus. While adhesion differed using the wild-type strain, this was not the case for the Fhb-deficient mutant, demonstrating that adhesion to the Gb3 of hCMEC/D3 by *S. suis* requires Fhb. However, the residual adhesion capacity in the absence of Fhb (30 to 50%) in non-treated and PPMP-treated cells also suggests that adhesion to BMECs is multifactorial. Indeed, a variety of virulence factors have been described to be implicated in the interactions with these cells, including the sortase A, D-alanylation of the lipoteichoic acid, enolase, and muramidase-released protein. Alongside adhesion, Kong *et al.* demonstrated that Fhb is also involved in Gb3-dependent monolayer traversal using a competitive inhibition assay. Consequently, the role of the *S. suis* Fhb in adhesion to and traversal of a human BBB model is Gb3-dependent.

Though the involvement of the *S. suis* Fhb in the adhesion to and traversal of a human BBB model is Gb3-dependent, the role of this interaction in the development of meningitis remained unknown. Firstly, Kong *et al.* created Gb3-deficient mice using the TALEN-mediated knockout method. Following systemic infection of mice, Gb3 was shown to be detrimental to host survival since Gb3-deficient mice succumbed significantly less to septic shock than did their wild-type counterparts using both the highly virulent strain 05ZYH33 and the classical virulent European strain 735. However, this negative role of Gb3 during the systemic infection was not attributed to uncontrolled systemic bacterial burden since quantities of bacteria in blood, spleen, and liver were similar between wild-type and Gb3-deficient mice. Since *S. suis*-induced host death during the systemic infection may, alongside uncontrolled bacterial burden, be the result of exacerbated production of inflammatory mediators, it could be hypothesized that Gb3, through a yet unknown mechanism, might be implicated in exacerbation of host inflammation.

Typical of the *S. suis* infection, individuals who survive the systemic infection are susceptible of developing a CNS infection characterized by meningitis. However, Gb3-deficiency significantly reduced development of meningitis since only wild-type mice presented moderate to severe signs of meningitis, as supported by histopathology and clinical scoring. This contrasting difference in clinical outcome was the result of brain bacterial burden, of which levels were higher in wild-type mice. As such, not only does Gb3 promote the development of *S. suis*-induced host death during the systemic infection, but it also increases susceptibility to meningitis. The similar blood but higher brain bacterial burdens suggest that Gb3 might be involved in *S. suis* traversal, which is also supported by the results obtained using the hCMEC/D3 monolayer. Alongside, Kong *et al.* demonstrated an important hemorrhage in the brain parenchyma of wild-type mice following infection with *S. suis*, which indicates that entry through the BBB, from where the parenchyma is easily accessible, occurred. In fact, brain parenchymal hemorrhaging is associated with *S. suis*-induced meningencephalitis as observed in the mouse models of infection.

As mentioned, the important brain parenchymal hemorrhaging observed in wild-type mice suggested that *S. suis* might induce an increase of the BBB permeability. By measuring the transepithelial electrical resistance and permeability of hCMEC/D3 to Lucifer yellow, which are assays routinely used to evaluate barrier permeability, Kong *et al.* demonstrated that the Fhb is indeed responsible for increased paracellular permeability. Of the cellular components involved in regulation of this phenomenon is the myosin light chain 2 (MLC2), of which phosphorylation is associated with increased endothelial permeability. In accordance, *S. suis* induced MLC2 Ser-19 phosphorylation in a Fhb-Gb3 dependent manner; meanwhile the Fhb-deficient mutant strain was unable to phosphorylate MLC2 at this site. As such, binding of the *S. suis* Fhb to Gb3 activates a signaling pathway leading to phosphorylation and activation of MLC2. Of the different intracellular components involved in this pathway are the myosin light chain kinase (MLCK) and the Rho-associated protein kinase (ROCK). Using inhibitors of these two kinases, Kong *et al.* demonstrated that ROCK, but not MLCK, was involved in *S. suis*-induced MLC2 phosphorylation. However, the exact pathway activated by binding of Fhb to Gb3 requires further investigation to fully understand the involved mechanisms.

In conclusion, using an *in vitro* human BBB model and Gb3-deficient mice, Kong *et al.* demonstrated,
through a variety of complementary approaches, how the S. suis Fhb may act as a key to unlock the blood-brain barrier and allow access to the central nervous system via binding to Gb3, thus participating in the development of meningitis. The mechanisms proposed in their article will not only increase our knowledge regarding the development of S. suis meningitis, but may also offer new insights into countering this pathogen. Indeed, since Fhb-Gb3 interaction appears important for the development of meningitis by S. suis, Kong et al. propose that Fhb could serve as a target for anti-adhesion therapies. Though future studies will be required to further our understanding of the Fhb, its role in meningitis, and potential synergism with other S. suis virulence factors, this study has paved the road for more extensive research to come.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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