Muramidase-released protein of \textit{Streptococcus suis}: New insight into its impact on virulence

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The Gram-positive bacterium \textit{Streptococcus suis} (\textit{S. suis}) is an important porcine pathogen and responsible for significant economic losses to the food industry, due to causing diseases in pigs, such as sepsis and meningitis. Further, as a zoonotic agent, \textit{S. suis}, especially strains of serotype 2, are a major concern due to the capability of equally causing disease in humans.\textsuperscript{1,2} Especially in south-east and east Asian countries \textit{S. suis} is a primary cause of bacterial meningitis. Large outbreaks occurred during 1998 and 2005 in China, which qualified \textit{S. suis} to be an important emerging or reemerging zoonotic pathogen on the rise.\textsuperscript{3,5}

\textit{S. suis} is a genetically highly diverse species, which is evidenced by the existence of multiple sequence types (STs).\textsuperscript{6,7} Throughout the years many potential virulence factors of \textit{S. suis} have been identified. The best characterized is the bacterial polysaccharide capsule, which arguably presents a critical virulence factor, since unencapsulated strains are eliminated by the host immune system and do not induce disease. In contrast, other proposed virulence factors of \textit{S. suis} are much less defined. Noteworthy, their impact during infection can strongly depend on the experimental infection model used for characterization.\textsuperscript{7-9} Besides gaining insight into the process of pathogenesis, identification and characterization of virulence factors of \textit{S. suis}, especially of those that are associated to the surface or secreted, is also of high importance for the development of vaccination strategies against this pathogen.\textsuperscript{5,9,10}

One proposed virulence factor of \textit{S. suis} serotype 2, whose role during infection was recently even described as “mystic,” is the Muramidase-released protein (MRP). MRP is a protein anchored to the cell-wall existing in a variant of 136-kDa as well as variants of larger and smaller sizes, which can be released into the culture supernatant during bacterial growth. Although, dependent on the strains taken into consideration, the presence of MRP appears to be associated with virulence, its lack does not necessarily prevent this characteristic of a given \textit{S. suis} strain, indicating that MRP is not required for full virulence.\textsuperscript{7-9,11,12} Likewise, the potential mechanisms of how MRP might mediate virulence during infection are a topic of ongoing research. A part of MRP displays some sequence similarity to a part of the fibronectin-binding protein from \textit{Staphylococcus aureus}, but binding to human fibronectin was not found for a \textit{S. suis} serotype 2 strain of ST1.\textsuperscript{13} On the other hand, newer studies with a Chinese serotype 2 strain of ST7 demonstrated the interaction of MRP with human fibronogen, which promoted disease in a mouse meningitis model and enhanced survival of \textit{S. suis} in human blood.\textsuperscript{14-16}

In this issue of \textit{Virulence}, Li and coworkers investigate the role of MRP by focusing on a variable region, which is conserved between 8 high virulent strains (including another serotype 2 ST7 strain from China) and also between 4 low virulent strains, but differs between these 2 groups.\textsuperscript{17} When comparing this domain (MRP-D1) from the high virulent serotype 2 ST7 strain to the equivalent region (MRP-D1”) from a low virulent strain, they find that MRP-D1, but not MRP-D1”, plays a role during the adherence to host cells. MRP-D1 also presented stronger \textit{in vitro} binding to human fibronectin, factor H, and fibrinogen or porcine IgG compared with MRP-D1”, whereas a region of MRP conserved in all analyzed \textit{S. suis} strains (MRP-D2) failed to interact with these host components. These data confirm some previous observations, but also contradict others, since, as mentioned above, binding of MRP to fibronectin was not observed in earlier studies.\textsuperscript{13,18} As the authors point out, low abundance of MRP in cell wall samples might
provide an explanation for not detecting this interaction in at least some experimental settings. Still, the results point to a contribution of MRP-D1 to S. suis serotype 2 virulence by means of different mechanisms, possibly involving adhesion, invasion and immune escape.

To gain better insight into a possible role of MRP-D1 during virulence, Li and colleagues make use of deletion mutants of the high virulent strain lacking either MRP-D1, MRP-D2 or complete MRP, which all display decreased binding to human epithelial cells, further supporting a function of MRP during adhesion. Deletion of MRP-D1, but not of MRP-D2, decreases the survival rate of S. suis in mouse RAW264.7 macrophages, and reduced survival of S. suis lacking MRP-D1 and full length MRP is also observed in pig blood. In a mouse infection model, the bacterial counts of the MRP-D1-deleted strain are reduced compared with the other strains in the blood and the brain of intraperitoneally challenged mice, and deletion of full length MRP or of MRP-D1 in S. suis leads to a higher survival rate of infected mice. The results of Li and coworkers agree with recent data, which identified MRP of a serotype 2 ST7 strain as human fibrinogen-binding protein with anti-phagocytotic properties, thus enhancing the survival of S. suis in human blood.14,15 Additionally, in vivo MRP contributes to the development of meningitis, and, as also observed by Li et al., causes an increase of bacterial loads in the brain of mice.16 Expression of MRP also contributes to an increase in blood-brain barrier (BBB) permeability in infected mice, and the interaction between MRP and human fibrinogen supports adherence and transmigration of S. suis across a human blood-brain barrier model in vitro.16 Similarly, MRP-D1 promotes binding of S. suis to a murine in vitro model of the BBB, collectively pointing to an involvement of MRP during brain invasion via the BBB. Interestingly, histopathological analyses of experimentally infected pigs, as well as studies in a mouse model, have provided evidence that the blood-cerebrospinal fluid barrier (BCSFB) at the level of the choroid plexus serves as entry site for S. suis into brain.19 It is possible that binding of MRP to fibrinogen also plays a role during adhesion and brain invasion of S. suis at the BCSFB, e.g. when interacting with the choroid plexus epithelium.

MRP has been described as a highly immunogenic protein and is, therefore, supposed to be expressed during infection in vivo. Due to this characteristic, MRP was suggested as a putative vaccine candidate, but a correlation between production of antibodies against MRP in pigs and protection against infection cannot always be noted.9,10,20,21 Li et al. now show that recombinant MRP-D1 and MRP-D2 induce serum antibody responses in mice, and immunization with MRP-D1 or MRP-D2 confer 50% and 12.5% protection to mice, respectively. Interestingly, in a killing assay using porcine whole blood, pre-incubation of S. suis with an serum against MRP-D1, but not one against MRP-D2, significantly decreased bacterial survival, indicating that MRP-D1 might exhibit vaccine potential.

The identification of a MRP domain conserved between either high virulent (MRP-D1) or low virulent (MRP-D1*) S. suis serotype 2 strains, which interacts with host factors and is involved virulence, provides new insight into the role of MRP during infection. At least in some strains MRP-D1 might be required for full function of MRP and represent an important virulence factor. Importantly, MRP-D1 offers vaccine potential. Still, the narrative of MRP-mediated virulence contains several unsolved questions. To answer these, Li and coworkers propose to (i) confirm their results in pigs in vivo, (ii) perform an exchange of MRP-D1 and MRP-D1*, respectively, between high and low virulent strains, and (iii) resolve the structures of MRP and its domains. The outcomes of these studies should be of high interest and help to further clarify the impact of MRP on virulence.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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