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Spotlight

New Virulence Factors Identified in Pneumococcal Meningitis

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Streptococcus pneumoniae causes bacterial meningitis with a high fatality rate globally. Patients who survive have a significant risk of lasting disabilities. Schmidt *et al.* have identified bacterial proteins that play a crucial role in pneumococcal meningitis: AliB, part of an oligopeptide transporter, and ComDE of the competence regulator.

Pneumococcal meningitis occurs when the normally nasopharynx-dwelling bacterium *S. pneumoniae* breaches the blood–brain barrier, triggering an inflammatory response and brain damage. The outcome for the patient is often catastrophic, with mortality rates of up to 55% [1] and neurological sequelae such as hearing loss and learning difficulties in up to half of the survivors [2]. Current vaccines are limited to targeting the polysaccharide capsule of 23 or fewer serotypes of the approximately 100 serotypes known. This means that new vaccine targets, ideally proteins common to all pneumococci, are actively being sought. Treatment of pneumococcal meningitis must take into account that some strains are antibiotic resistant, whilst lysis of susceptible pneumococci releases bacterial factors, including the toxin pneumolysin (Ply), causing a massive inflammatory response which exacerbates brain damage. In addition to Ply, several virulence factors have been identified as contributing to meningitis by aiding brain invasion, or bacterial survival, or altering the immune response. These include PspC/CbpA, PavA, NanA, RrgA, the polysaccharide capsule, and the peptidoglycan and lipoteichoic acid of the cell wall. Although gene expression studies have given us much useful information about potential virulence factors at different anatomical sites, in their recent paper, Schmidt *et al.* use a proteomic approach to identify novel factors associated with meningitis *in vivo* to increase understanding of the mechanism by which pneumococci cause meningitis [3].

This new study reveals proteins not previously considered to be virulence factors in meningitis as playing a crucial role in the severity of the disease, by comparing the proteome of pneumococci isolated from the cerebrospinal fluid (CSF) of mice with meningitis to that of pneumococci *in vitro*. To be able to analyze the pneumococcal proteins present in the CSF, which must be a complex mixture of host and bacterial proteins, they first made a library of pneumococcal proteins expressed under various conditions. This elegant approach allowed them to identify the pneumococcal proteins with confidence despite the relatively low numbers of bacteria recovered from the CSF. They found expression of the proteins ComD, ComE, and AliB in the *in vivo* samples but not in the *in vitro* control. Interestingly, these are all proteins involved in the sensing and response to specific peptides.

ComD is a transmembrane histidine kinase that autophosphorylates upon binding to competence-stimulating peptide (CSP). This sensing of the extracellular level of CSP is the way that pneumococci regulate competence for natural genetic transformation during quorum sensing [4]. The phosphoryl group is transferred to ComE, which is the response regulator that stimulates transcription of many genes involved in competence.

AliB is a substrate-binding protein of an oligopeptide transporter, named Ami-AliA/AliB, proposed to be involved in sensing by the pneumococcus of its environment [5]. This ATP-binding cassette (ABC) transporter consists of two permease domains: AmiC and AmiD, two ATP-binding domains, AmiE and AmiF, providing the energy for transport, and three substrate-binding proteins AmiA, AliA, and AliB ('Ali' is derived from 'Ami-like') to bring the peptides to the permease for uptake into the bacteria. Binding of AliB to its specific peptide ligand AIQSEKARKHN, a sequence found in other bacterial species, triggers changes in pneumococcal phenotypes, including increased growth, bacterial chaining, and a decrease in transformation rate *in vitro* [6,7]. AliB has previously been shown to have a role in colonization, in line with its putative role in detecting other bacterial species in its niche, but not in invasive disease [8].

To test whether the proteins expressed in CSF really play a role in meningitis, Schmidt *et al.* infected mice intracisternally with wild-type pneumococci or mutants lacking functional ComDE, AliB, or both. The mutants' ability to induce meningitis was impaired compared to the wild type as determined by white blood cell count in the CSF, clinical score, and the number of cortical hemorrhages, although there was little difference in levels of the cytokines IL-1β and CXCL2 or the number of pneumococci in the brain.

In addition to the Ami-AliA/AliB transporter, nonencapsulated pneumococci very often have, in place of the capsule operon, two genes named *aliB-like* ORF 1 and *aliB-like* ORF 2 due to their homology to *aliB* (referred to as *aliC* and *aliD* by Schmidt *et al.*) which also bind specific foreign peptides [9]. Nonencapsulated pneumococci do have AliB

yet generally do not cause meningitis, indicating that it is the capsule which is the critical factor for meningitis. The authors also note that the mutant lacking AliB has a lower titre in blood than the wild type, which is an interesting parallel to the finding that *aliB-like* ORF 1 and *aliB-like* ORF 2 (*aliC* and *aliD*) also increase pneumococcal survival in chinchilla blood [10].

Having shown that ComDE and AliB are crucial for meningitis in their mouse model, it would be intriguing to uncover the exact mechanism, as the authors find that the mutants do not have altered growth, susceptibility to oxidative stress, or expression of other virulence factors such as capsule or pneumolysin. Whether it is a matter of nutrition or uptake of specific peptides for signaling is still an open question.

Since meningitis is a dead-end outcome for the bacteria, ComDE and AliB have presumably evolved to perform their functions at other sites, particularly the nasopharynx. The novel proteomic library made by Schmidt *et al.* would be a valuable tool to look for expression of ComDE and AliB as well as other proteins at the different relevant anatomical locations. Uncovering which bacterial proteins are expressed where will be valuable information for the design of future vaccines.

This paper thus opens up the field of protein expression research in pneumococci and paves the way to learn about the expression of proteins, including ComDE and AliB, in human meningitis.

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