Overview

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The pneumococcus: 'old man's friend' and children's foe

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Pneumococcal lower respiratory tract infection is the final pathophysiological episode in many human lives. This is attested by the old age adage in the title above (attributed to Canadian physician Sir William Osler) and the high frequency at which respiratory infection is listed as the primary cause of death on death certificates (<http://www.statistics.gov.uk/StatBase/ssdataset.asp?vlnk=7433&Pos=&ColRank=2&Rank=480>). A major recent breakthrough in reducing the burden of disease caused by the pneumococcus (*Streptococcus pneumoniae*) has been the implementation, in several Western countries, of a protein-polysaccharide conjugate vaccine designed, not for the elderly, but for the paediatric age group. Young children have similarly high rates of serious pneumococcal disease as the elderly (http://www.hpa.org.uk/infections/topics_az/pneumococcal/IDU_popu_age.htm; McIntosh, 2003). Although middle-ear infection (otitis media) in the under fives is extremely common, costly in morbidity, and adds significantly to the use of antibiotics in the community (and thus to the emergence of antibiotic resistance), it is acute respiratory infection in children that accounts for the main toll of the pneumococcus on children's lives (http://www.who.int/vaccine_research/documents/new_vaccines/en/index2.html).

On these counts it might be tempting to look for strategies to 'do a smallpox' and eliminate this bacterial scourge of human lives. However, the pneumococcus is usually not a pathogen at all, but a commensal of the upper respiratory tract, colonizing 20–50 % of young children in Western Europe and up to 90 % of young African infants (R. Adegbola, personal communication) in cross-sectional studies. Immunization with conjugate vaccines dramatically reduces detectable carriage rates of the pneumococcal serotypes covered by the vaccines used (Klugman, 2001). However, overall rates of carriage are little altered, because it seems that other serotypes become detectable in their place (Veenhoven *et al.*, 2004). Perhaps, therefore, one should hesitate before intervening to change carriage patterns, and the acquisition of natural immunity to a bacterial species that, it would appear, has been colonizing many human populations for a very long time. Among the various capsule serotypes, of which there are at least 90, there is not a straightforward relationship between frequency of detected carriage and frequency of invasive disease. For example, invasive serotype 1 strains (which are not covered in the

currently licensed conjugate vaccine) are often found in blood and empyema fluid, but are rarely detected as being carried in the nose. With growing evidence that interactions between different upper respiratory microbial species can influence colonization and invasive disease (Bogaert *et al.*, 2004; Lysenko *et al.*, 2005), the possible implications of making changes to the nasal 'Petri dish' become more scary.

However, these various concerns and conundrums should not detract from current achievements. The USA is now 5 years into universal immunization using a seven-valent conjugate vaccine, and the impact has been impressive. Not only have overall rates of pneumococcal invasive disease fallen dramatically, but rates among black Americans (historically much higher than among whites) have also dropped to equally low levels, ironing out this racial predilection to cause disease. Impressive and unquestionable falls in disease rates among young adults (parents) and older adults (grandparents) are an additional huge benefit (Whitney *et al.*, 2003). Despite these successes, there is awareness that we still understand comparatively little about the pneumococcus as a commensal and pathogen. It is thus anticipated that new advances in molecular genetics, functional genomics and molecular immunology may lead the way forward in the future to more successful measures for control, and ultimately prevention, of pneumococcal disease.

The pneumococcus has been researched in pure culture by microbiologists for over 80 years. It was, of course, the basis of the discovery by Griffiths in 1928 of the transforming principle which converted non-infective rough (uncapsulated) colony-forming diplococci to virulent smooth (capsulated) forms and was later identified by Avery, MacLeod and McCarthy as DNA. While the pneumococcal capsule is an essential virulence factor, it is now recognized that there are plethora of additional cell-surface molecules orchestrating pneumococcal pathogenesis, in addition to the toxic activities of pneumolysin and the inflammatory effects of cell wall fragments. Cell-surface proteins have been identified as key players in pneumococcal adhesion, colonization and virulence. In Gram-positive bacteria, there are two general mechanisms by which proteins are anchored at the cell surface. One involves an amino-terminal lipid modification, sheathing the protein to the outer leaf of the cytoplasmic membrane. The other involves recognition of

a carboxyl-terminal motif by a sortase enzyme that links the polypeptide to cell wall peptidoglycan. In the pneumococcus, and closely related strains of *Streptococcus mitis*, a special class of cell-surface proteins designated choline binding proteins (Cbps) are additionally produced. These contain repeat blocks of amino acids that secure the proteins to the choline-containing lipoteichoic acid cell-surface polymer. The Cbps are deeply implicated in influencing pneumococcal colonization of the nasopharynx, in manipulation of the host defences (Bergmann & Hammerschmidt, 2006), and in regulation of virulence (LeMessurier *et al.*, 2006). It is believed that the Cbps have the potential to be useful as future vaccine components, although significant antigen variability across strains currently seems to be a compounding factor.

A major attraction of the pneumococcus to microbial geneticists over many decades has been that it is naturally transformable. This has provided a basis for numerous fundamental studies of DNA uptake, genetic recombination mechanisms, and population dynamics in bacteria. The discovery by Håvarstein *et al.* (1995) that the extracellular molecule stimulating development of competence for DNA-mediated transformation was an unmodified secreted peptide (competence-stimulating peptide, CSP), was a seminal event in pneumococcal research. It meant that virtually all pneumococcal isolates could be genetically manipulated, and this led to a massive surge in interest and progress in pneumococcal genetics. More recently, it has been firmly established that an early event following stimulation of streptococcal cells by CSP is the expression of a novel sigma factor that controls not only later recombination-associated events but also, in related streptococcal species, biofilm formation. So finely tuned is the development of competence that overexpression of the two-component signal transduction system (ComDE) that recognizes CSP prevents development of spontaneous competence (Guiral *et al.*, 2006a). The intricacies of signal-mediated gene regulation are further exemplified by the new finding that LuxS, the enzyme involved in production of the putative universal quorum-sensing molecule autoinducer 2 (AI-2), negatively controls competence in the pneumococcus (Romao *et al.*, 2006). The development of new genetic techniques is fundamental to such studies and two significant advances (Guiral *et al.*, 2006b; Kloosterman *et al.*, 2006) for gene expression analyses hold much promise for gaining new insights into protein functions. Novel molecular analytical methods will be paramount in the future for characterizing the approximately 30% of total open reading frames present within various sequenced pneumococcal genomes (Tettelin *et al.*, 2001) (<http://www.sanger.ac.uk/Projects/Microbes/>) for which no functions or homologies are currently assigned. Among these proteins may be new targets for drug development, or for incorporation into vaccines.

So what is the future for the pneumococcus? More widespread use of pneumococcal vaccines in children in countries that can afford them seems certain in the short term.

However, use of conjugate vaccine will undoubtedly result in shifts in pneumococcal epidemiology, as evidenced by the recent emergence of invasive disease due to serotype 19A in the USA (Hicks *et al.*, 2005). These effects will, in turn, necessitate reformulation of the vaccines in use. If significant changes in disease rates due to other respiratory tract bacterial species are also observed, then this will raise questions as to whether pneumococcal immunization and alteration to carriage patterns are responsible. In the end, the most logical approaches appear to be to: (1) seek a better understanding of natural immunity which, one must assume, is responsible for the lower rates of carriage and disease in older children and young adults; and (2) identify on the basis of molecular and pathogenicity studies new targets for drug development and for circumventing inadequacies as they arise with current vaccines. In this knowledge must lie the key to rational strategies for control of pneumococcal disease in the future, particularly in those poorer countries that carry the greatest burden.

The papers collected in this special issue include two up-to-date reviews on important topics related to pneumococcal molecular biology. They also include exciting new developments in understanding aspects of population structure, virulence gene expression, and gene regulation, and describe new tools for genetic analysis in pneumococci. The pneumococcus might be a friend (of sorts!) for the aged, and a foe for the young, but it is a dream for the microbiologist!

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