Pneumococcal polysaccharides – Advancing research and development

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Carbohydrates in the form of capsular polysaccharides and lipopolysaccharides constitute a major component of the cellular surface for many bacterial species[1]. In *Streptococcus pneumoniae*, capsular polysaccharides function as major virulence factors that protect the bacterium from phagocytosis by host immune cells. Within this species, capsular polysaccharides exhibit enormous structural diversity, resulting in significant differences in immunogenicity and antigenicity. In turn, this has presented numerous challenges toward the prevention and serological analysis of pneumococcal infections[2]. In the present article, we will discuss the importance of capsular polysaccharides in *S. pneumoniae* infection, and will describe the use of purified serotype-specific polysaccharides in the development and evaluation of pneumococcal vaccines, the verification of novel immunoassays, and tracking bacterial disease epidemiology.

S. pneumoniae is a major human pathogen known to cause both invasive and non-invasive infections such as pneumonia, meningitis, bacteremia, otitis media, and sinusitis[2,3]. More importantly, this bacterium is considered to be the leading cause of vaccine-preventable morbidity and mortality worldwide. A recent report estimated that pneumococcal disease was responsible for 445,000 hospitalizations and 22,000 deaths within the United States in 2004, corresponding to approximately $3.5 billion in direct medical costs[4]. Moreover, the World Health Organization (WHO) estimates that *S. pneumoniae* annually contributes to the death of more than one-million children under the age of five years, globally, which is more than malaria, tuberculosis, and AIDS combined[5].

This pneumococcal pathogen causes disease through the production of numerous virulence factors, including pneumolysin, pneumococcal surface proteins, and capsular polysaccharides[2]. Of these, the capsular polysaccharides – which are polymeric, surface-exposed carbohydrate molecules that encapsulate the bacteria – are considered to be the most important virulence factors as they shield the bacteria from neutrophil clearance. Currently, more than 90 different capsular serotypes have been identified, each distinguishable by serological response, variations in chemical structure, and related genetic mutations[2,6-11]. These unique differences can associate with distinct epidemiological properties, including variations in carriage or prevalence of disease. For example, capsular types with higher ratios of charge to carbon may be physically larger, making them more resistant to neutrophil clearance[12,13].

Because of their importance in pneumococcal pathogenicity, capsular polysaccharides have been components in a number of serotype-specific vaccines. In 1983, a 23-valent pneumococcal polysaccharide vaccine (PPV23, Pneumovax 23; Merck) was developed which contained the pooled capsular polysaccharides purified from 23 different serotypes[14]. Due to poor immunogenicity in infants, PPV23 was not approved for use in children younger than 2 years of age. In 2000, after nearly two decades without a suitable vaccine for juveniles, a 7-valent pneumococcal conjugate vaccine (PCV7,

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Prevnar; Wyeth) was developed and licensed for use in infants and young children in the United States. This vaccine was created through the covalent coupling of a protein carrier to the purified polysaccharides from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F[15]. However, to extend vaccine coverage to additional serotypes, a 13-valent pneumococcal conjugate vaccine (PCV13, Prevnar 13®; Pfizer) was later licensed for pediatric and adult use. PCV13 is composed of the same serotype-conjugates included in PCV7, as well as polysaccharide conjugates for serotypes 1, 3, 5, 6A, 7F, and 19A[16].

In addition to their role in pneumococcal vaccine development, purified polysaccharides are also necessary for evaluating how effectively a vaccine can induce an immune response. Currently, immunogenicity is measured using a standardized enzyme-linked immunosorbent assay (ELISA) protocol designed for the quantitation of human IgG antibodies specific for *S. pneumoniae* capsular polysaccharides. The protocol for this assay, which was developed in 2000 by representatives from academia, government, industry, and the WHO, is provided as a training manual containing the standard operating procedures for the preparation, execution, and analysis of the ELISA[17]. In this assay, ELISA plates are first coated with purified, serotype-specific capsular pneumococcal polysaccharides (ATCC, Manassas, VA). Following adsorption of the individual polysaccharides, dilutions of absorbed human sera are then added to the ELISA plates. The serotype specific antibodies that remain bound to the purified pneumococcal polysaccharides are then detected and quantitated using an anti-human IgG antibody conjugated with alkaline phosphatase[17].

In recent years, capsular polysaccharides have been similarly used in the development and evaluation of novel immunoassays. Though the WHO ELISA protocol provides a standardized, accurate method for the evaluation of vaccine efficacy, it has recently come under scrutiny as it is laborious and consumes large volumes of serum. To this end, the development of novel multiplex immunoassays that allow for the simultaneous measurement of specific antibodies against various pneumococcal polysaccharides have been explored[18-20]. For example, a recent study by Klein *et al.* described the development and characterization of a multiplex bead-based antibody quantification assay (MBIA) based on Luminex® xMAP® technology (Luminex, Austin, TX) that required the conjugation of purified serotype-specific capsular pneumococcal polysaccharides (ATCC, Manassas, VA) to fluorescent beads. In this assay the polysaccharide-bead conjugate mixture was incubated with various dilutions of pre-adsorbed serum. Following incubation, bound antibodies were quantified through the use of phycoerythrin-labeled goat anti-human reporter antibodies[18]. As compared to the traditional ELISA protocol, the MBIA procedure was able to provide a more efficient, cost-effective means of analyzing small volumes of serum; though, careful optimization, stringent validation, and continuous monitoring are still required.

Along with their function in detecting and enumerating anti-pneumococcal antibodies, the combinatorial use of capsular polysaccharides in immunoassays is ideal for tracking the epidemiology of serotype-specific pneumococcal infections following vaccination. The gold-standard method for tracking invasive *S. pneumoniae* infection has occurred through traditional culture-based assays. However, good-quality samples are not always available, culturing and serological analyses can be fairly time-consuming and laborious, and the detection of viable pneumococci can be hampered by use of antibiotics[21,22]. Furthermore, serotype-specific assays to diagnose community acquired pneumonia caused by *S. pneumonia* only recently became available[23,24]. To assess the positive impact of the 13-valent pneumococcal conjugate vaccine (PCV13, Prevnar 13®; Pfizer), serotype-specific diagnostics can provide valuable data on how effectively the vaccine reduces disease in the population, as well as assist in monitoring for the potential emergence of rare serotypes.

Pfizer, a global leader in the biopharmaceutical industry committed to producing quality products that make an impact on health worldwide, is collaborating with ATCC, a leading provider of standards for the global scientific community, to ensure that researchers worldwide have access to purified, high-quality polysaccharides. The supply of pneumococcal polysaccharides is essential in continuing to drive pneumococcal research, predicting future epidemiological shifts, and combating this deadly pathogen.

Overall, capsular polysaccharides are bacterial antigens that play a significant role in pneumococcal pathogenicity. By targeting these polymeric carbohydrates through the use of polysaccharide or polysaccharide conjugate vaccines, the disease burden of *S. pneumoniae* serotypes covered by these vaccines has been reduced significantly. Availability of purified pneumococcal polysaccharides has also facilitated the development of a number of novel technological advances in the development of serological assays and diagnostics, and the understanding of disease epidemiology.

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