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Pneumococcal Surface Protein A: A Promising Candidate for the Next Generation of Pneumococcal Vaccines

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ABSTRACT

Streptococcus pneumoniae is the bacterium that causes pneumococcal disease which often results in pneumonia, meningitis, otitis media, septicemia and sinusitis. Pneumonia, particularly, is a significant cause of worldwide morbidity and a global health burden as well. Treatment often relies on antimicrobials, to which the pathogen is frequently mutating and rendering infective. Consequently, vaccination is the most effective approach in dealing with pneumococcal antimicrobial resistance (AMR). Unfortunately, the current pneumococcal polysaccharide and conjugate vaccines have a narrow serotype coverage. Therefore, the current need for vaccines with a broader serotype coverage cannot be overstated. Pneumococcal Surface Protein A and C are potential vaccine candidate antigens present in over 90% of the strains from clinical isolates as well as laboratory non-encapsulated strains. Pneumococcal Surface Protein A is an active virulent factor that pneumococci use to evade complement-mediated host immune responses and has been shown to elicit immune responses against pneumococcal infections. This review explores the potential utilization of Pneumococcal Surface Protein A to immunize against *S. pneumoniae.*

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Introduction

S. pneumoniae is a gram-positive bacterial pathogen that causes pneumococcal disease in humans. Infections of *S. pneumoniae* often result in pneumonia (the most common complication), meningitis, otitis media, septicemia, and sinusitis (1,2). Pneumococcal infections are considered a heavy global burden on the health systems, causing the highest number of deaths among infectious diseases (3). In normal individuals, the bacteria may be part of the normal human microbiome and reside asymptomatically in colonies in the upper respiratory tract and nasopharynx (4,5). However, any chance accorded to the pathogen, especially in immunocompromised persons, children and the elderly, results in invasive infections As of 2008, the Centers for Disease Control (CDC) projected approximately 1 million yearly deaths of children below the age of five resulting from pneumococcal infections, in the US alone (6).

Over a century of studying *S. pneumoniae* has led to a substantial understanding of the pathogen's physiology, pathogenesis, and immunity. Consequently, numerous antibiotics have been developed against the pathogen, and remain the mainstay of treatment in pneumococcal infections (7). The primary anti-pneumococcal drugs are β-lactams, macrolides and fluoroquinones (8). The tolerance of *S.*

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pneumoniae to macrolides and β-lactams is a significant global concern. Research has shown that AMR in pneumococcus occurs in over 20% of the cases in some countries in Europe (9,10). The development of AMR in pneumococci has been linked to genetic transformation and selection of resistant pneumococci during asymptomatic carriage in children, who are often exposed to antibiotics. Therefore, restriction of fluoroquinolones' use in children explains why resistance rates against it remain relatively lower (11,12).

According to Castiglioni (2019), vaccination has long been regarded as the most impactful discovery in medicine from the public health perspective; however, it was not until recently that it was considered as the best solution to AMR. A publicly commissioned study in the UK dissected the topic deeply and reported the scientific and economic benefits of using immunization to fight AMR (13). There are two kinds of vaccines that are currently used to elicit immunity against *S. pneumoniae*: pneumococcal conjugate vaccines, and polysaccharide vaccines. PPSV23 is a 23-valent pneumococcal vaccine that protects against 23 capsular serotypes of the bacteria in individuals aged 2 years and above: however, it does not protect children below the age of 2. The 7-valent PCV7 was developed to protect against the 7 most common serotypes causing invasive pediatric pneumococcal disease. The most recent vaccine, PCV13, protects against 13 serotypes, in both adults and children (14-17). Daniels et al. (2016) proposed that the development of new and more virulent serotypes that are not protected by the vaccines reinforces the need to research into novel vaccine antigens (18).

The Pneumococcal Surface Protein A (PspA) is a cell-wall-associated antigen in *S. pneumonia*. It is a key virulent factor utilized by the pathogen to bind human lactoferrin and interfere with the opsonization of the bacteria (19,20). Roberts et al. (2019) have demonstrated that the protein offers broader protection against pneumococcal infections than the current pneumococcal conjugate vaccines (21). Yatim et al. (2013) observed PspA proteins in 95% of the strains affecting Malaysian children (22). Several other extensive studies have revealed over 90% consistency of the antigen among pneumococcal strains (23-25). Such consistency, combined with its role in the pathogenicity of the bacteria, renders PspA a promising vaccine candidate. In this review, we explore the possible use of recombinant PspA as an antigen for immunization against *S. pneumoniae.*

Pneumococcal Virulence and Host Immunity

Pneumococci have a vast array of virulence factors (Fig. 1) that foster its attachment and invasion of host cells and allow it to evade the host's attempts to flush it out (26). A healthy and robust immune system is a prerequisite for flushing out pathogens before they are infective (27). Conversely, a weaker or incapable immune system provides a conduit for the attack even by the normally nonpathogenic microorganisms. One's immunity develops with age and gets stronger in adults, but it gets deteriorating in elderly people (28,29). *S. pneumoniae* exists asymptomatically in immunocompetent individuals, but those with weaker immune systems especially the children, elderly and immune-compromised persons have increased susceptibility to invasive infections resulting in pneumococcal disease (30,31).

The capacity of pneumococci to expand their genetic material through transformation and recombination is the primary technique for their virulence (32). The degree of genetic diversity within *S. pneumoniae* must be investigated in order to fully comprehend its pathogenicity as well as create viable therapies and vaccines (33). The virulence profile of several pneumococcal strains is optimized by variations in genetic content and single genes. Genome diversification was defined by Zhao et al. (2018) as bacterial ability to develop in a variety of host settings (34). Because of variations in the genetic content of their dispensable genes, genetic diversity has been found among identical clones of the pathogen. Dispensable genes aren't required for bacterial growth; rather they give the pathogen selection benefits like antibiotic resistance. Allele replacement introduces additional variations to the microbe's core genome. This is due to the bacteria's absence of SOS genes, which prevent it from repairing damaged DNA. Genetic variation can also be influenced by pregnancy. Chaguza et al., (2020) established a model to estimate carriage time and combined their results with whole-genome sequencing (WGS) data. In comparison to the patient's age and prior carriage, the WGS data showed that bacterial

genetic variation accounted for phenotypic variance (35).

Critical virulence factors in *S. pneumoniae* infections include: capsular and cell wall polysaccharides, choline-binding proteins (CBPs), Pneumococcal Surface Protein A (PspA), Pneumococcal Surface Protein C (PspC), pneumolysin, autolysin, among others.

Immune responses mounted against pneumococcal infections are mediated through both arms of the immune system: innate and adaptive (36) Innate immunity is mediated through the mucosa and respiratory epithelial cells, phagocytic cells and pattern recognition receptors (17, 37, 38). Adaptive immune responses, which are elicited a few days after the infection, are mediated through B and T lymphocytes (39). Pneumococcal-specific IgA antibody, secreted at infection sites, is essential for opsonizing the *S. pneumoniae* pathogens and promoting phagocytosis (40). IgA1 protease possessed by the pathogen, however, cleaves IgA and prevents opsonization. The binding of the Fab fragment to the cell wall following IgA cleavage exposes CBPs, lowers the capsular negative charge and causes the bacterial cells to attach more firmly (41-43). Simultaneous activation stimulates differentiation of naïve B cells into IgM-positive memory B cells and facilitates class switching to produce other Ig types required to flush out the infection (44).

Presentation of antigen peptide-MHC complexes by APCs stimulates T cell response. CD4 T helper cells are activated by co-stimulatory proteins and initiate a cascade of events geared towards generating both cell-mediated and humoral responses against the infection (45,46). Immune responses following pneumococcal infections are often hampered in immunocompromised individuals. Infant T cell responses fail because exposure to foreign antigens is often restricted before birth. The efficacy of adaptive responses also diminishes in aged individuals and they are thus susceptible to morbidity (47).

As already established, capsular polysaccharides and pneumolysin are among the primary pneumococcal virulence factors, and existing vaccines are based on these two (48). However, a few studies have described the existence of non-encapsulated pathogenic strains; and these are usually unaffected by the existing vaccines (49-51). This, alongside the discovery of new virulence factors and new methods of pathogenesis for existing virulence factors, demonstrates the pneumococcus' strength in the face of environmental obstacles, particularly those presented by antimicrobials and vaccines. Recent advancements in our grasp of pneumococcal virulence factors may open the door to the creation of innovative treatment or preventative methods.

Novel vaccine approaches

The main antigenic constituents of the present pneumococcal vaccines are capsular polysaccharides. However, polysaccharide-based vaccines are unable to protect children aged 2 years and below. Conjugate vaccines have therefore been developed by coupling immunogenic proteins with the capsules. These vaccines elicit responses efficiently, but only against a limited number of serotypes. The most recent development approaches, therefore, include using immunogenic proteins that are consistent in a wide array of serotypes (52).

Immunogenic proteins of *S. pneumoniae* that contribute to its virulence are an avenue for developing vaccines with a wider serotype coverage. Some of the proteins include PspA, PspC, PsaA, PppA, Zinc metalloprotease B, among others (53,54). Table 1 illustrates some of the pneumococcal vaccines that are currently in use and those that are still in clinical trials. PspA and PspC have been widely studied as critical pneumococcal virulence factors expressed by virtually all strains, and exhibit diverse organ-specific effects (55-57).

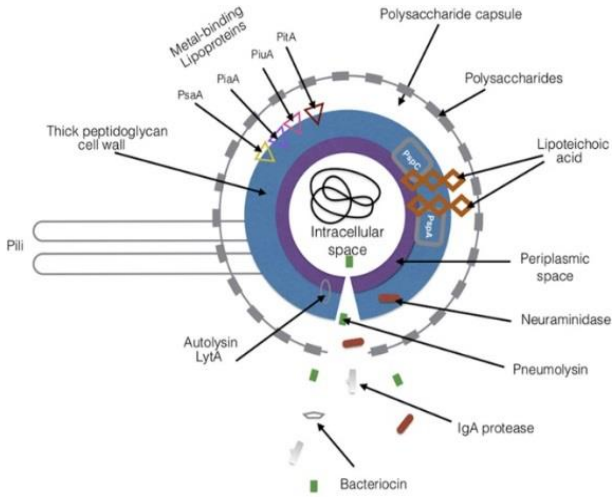

Figure 1. Common pneumococcal virulence factors (36).

Table 1. Current pneumococcal vaccines in use and vaccine candidates in clinical trials; in use (A) and in clinical trials (B)

S No.	Pneumococcal Vaccine Candidates	Type	Status
1	23-Valent Pneumococcal Polysaccharide Vaccine (PPV23)	Polysaccharide	A
2	Pneumococcal Conjugate Vaccine (PCV-7, PCV-10 and PCV-13)	Conjugate	A
3	Pneumococcal Conjugate Vaccine (PCV-15 and PCV-20)	Conjugate	B
4	Pneumococcal Protein Vaccine (PspA, PhtD, SktP and pneumolysin)	Protein	B

Pneumococcal Surface Protein A

PspA is a CBP protein that inhibits cell surface binding of C3 complement constituent thus interfering with opsonization (58). The protein is present in clinical isolates, as well as non-encapsulated strain Rx1. PspA was identified with the help of monoclonal antibodies that could elicit pneumococcal immune responses in mice (59). Immunizing CBA/N mice with congenic PspA+ and PspA- pathogens has proven that PspA elicits protective anti-pneumococcal antibodies (60). Insertional inactivation of the gene that codes for PspA in three *S. pneumoniae* strains has also been shown to reduce the virulence of all three, with two becoming completely avirulent (61,62). These observations are significant to future pneumococcal vaccine R&D, as CBA/D mice, like infants and elderly persons, are unresponsive to the current pneumococcal vaccines (63,64).

Following an extensive experimental study, Tu et al. (1999) reported that in mice infected with *S. pneumoniae*, PspA inhibits complement-dependent host immune responses mediated by factor B (65). Immunoblots of opsonized bacterial cells showed that C3b was present on PspA- bacteria, but not on PspA+ bacteria. In addition, the α-chain of C3b was cleaved and its processing was lowered by PspA. It can therefore be inferred that the virulence of PspA is by blocking the opsonic binding of C3b on the pathogen's surface or by inhibiting the functionality of the alternative pathway's C3 convertase. When the later mechanism is utilized, PspA lowers the quantity of C3b deposited on the bacterial surfaces, effectively inhibiting complement-mediated pathways of bacterial clearance (44,57,66).

Being a highly variable protein, PspA can be grouped into three families and six different clades based on the sequence of its amino end (67). The protein elicits immune responses against fatal septicemia resulting from different pneumococcal serotypes. The N-terminal of PspA comprises repetitive helices protruding from the cell surface. The region between the two terminals of the protein, consisting of 60-80 amino acids, is particularly rich in proline, and also highly variable in both length and sequence. Antibodies targeted against this portion of the protein are cross-reactive but not cross-protective (68,69).

Immunization with PspA

PspA is In mice models, the strongest immune response is elicited when the immunizing PspAs belong to a similar family as the challenge PspAs (70). This emphasizes the need to include proteins from different clades and families when developing PspA-based vaccines for protection in humans. Administering both PspA and IL-12 intranasal has been demonstrated to increase the secretion of systemic antibodies, enhance opsonization, and confer stronger protection (71,72). Consequently, IL-12 could be an effective adjuvant for PspA-based immunization. Also, a toll-like receptor 5 binding protein found in *Vibrio vulnificus*, FlaB, exhibits a high mucosal adjuvant activity. Experimental studies in mice have shown the potential role of FlaB in PspA immunization. In these experiments, one group of mice was immunized with recombinant fusion proteins made of both PspA and FlaB, the second group was immunized with a direct mixture of PspA and FlaB, while the control group was immunized with PspA alone. The findings revealed more protection in groups one and two than in the control group. However, group one elicited a much stronger immune response than the second group. we can therefore infer from these studies that genetic recombination of FlaB and PspA is necessary for the high efficacy of FlaB-adjuvanted *S. pneumoniae* vaccines (73).

Phase 1 clinical trials of a recombinant PspA vaccine belonging to family 1 have been completed in

man, and the vaccine is safe and immunogenic. In addition, when the immune human serum was administered to mice infected with pneumococcal pathogens expressing either PspA family 1 or 2, an immune response was elicited, but not as strong against family 2 as against family 1. This reinforces the need for using IL-12 or FlaB-adjuvanted recombinant pneumococcal vaccines (74).

PspC, also known as CbpA, is another vaccine candidate that has been extensively studied (75). PspC binds secretory IgA to enable it to bind the host's epithelial and endothelial cells. Immunizing mice with PspC protects against pneumococcal septicemia. Moreover, Daniels et al. (2010) showed that antibodies targeted against PspC exhibit cross-reactivity against PspA (76). This cross-reactivity has been attributed to the many similarities that exist between the two surface proteins (77). Both proteins have a proline-rich region in the α-helix between the N and the C terminals. Within the proline-rich regions of the two proteins, there exists an invariable non-proline block (NPB) with a sequence of thirty-three amino acids. Therefore, immunization with recombinant proline-rich molecules and monoclonal antibodies against the NPB or proline-rich epitopes elicits a robust immune response in mice (78,79). Because the proline-rich and NPB regions are highly conserved ad cross-reactive, they represent potential targets for PspA and PspC-based recombinant vaccines. Several studies have examined both PspA and PspC as active pneumococcal virulence factors, present consistently in over 90% of all *S. pneumoniae* strains, and a majority of them have demonstrated the importance of combining the two (62,80-82). Schachern et al. (2014) did extensive research on the immunization capabilities of PspA, PspC, and both PspA and PspC. From their findings, they were able to demonstrate that PspC has the ability to dramatically boost the efficacy of a multi-component PspA vaccine (83,84).

Conclusions

PspA is a critical virulence factor in pneumococci that acts by blocking complement-mediated opsonization of the pathogens. This prevents phagocytosis, effectively inhibiting clearance of the pneumococci. PspA has a greater capacity to elicit stronger immune responses with a broad serotype coverage than the current polysaccharide-based and pneumococcal conjugate vaccines. Polysaccharide-based vaccines do not offer protection to infants, while both of them have only limited serotype coverage and also offer zero protection against non-encapsulated strains. PspA antigens are present in over 90% of the pneumococcal strains, including non-encapsulated ones. Immunization targeting this antigen provides a wider serotype coverage and is thus more efficient. Pneumococcal vaccine R&D should employ a recombinant approach that integrates both PspA and PspC in a multi-component vaccine, because of their combined stronger immune response and even wider serotype coverage resulting from cross-reactivity. A combination of all the three families of the PspA antigen in the same vaccine is also recommended, to eliminate the negative effects of the phenotypic variations that could result from the existence of different PspA families. IL-12 and FlaB are efficient adjuvants that can be utilized to enhance the performance of PspA-based vaccines. However, FlaB-adjuvanted PspA vaccines would require genetic recombination of protein segments from both PspA and FlaB, rather than a mere mixture of the two, to function efficiently.

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