

## Pneumococcal vaccines: an update on current strategies

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### Abstract

*Streptococcus pneumoniae* is a major cause of morbidity and mortality in infants, children and the elderly. Despite the availability of excellent antimicrobial therapy and adequate health care systems, respiratory diseases and invasive infections caused by pneumococci still comprise a major health problem. The emerging resistance to penicillin and other commonly used antibiotics underscores the importance of the development of novel vaccine strategies to combat pneumococcal disease. Although the 23-valent polysaccharide (PS) vaccine is immunogenic and protective in most adults and children over 5 years of age, they fail to protect children under 2 years of age. Fortunately, the recent conjugate vaccines have shown to be highly efficacious in preventing invasive diseases in this risk group. Moreover, promising results regarding prevention of pneumonia and acute otitis media have been published. Unfortunately, protection is raised against a limited number of pneumococcal serotypes, and serotype replacement and subsequent vaccine failure have become a serious concern. Currently, several pneumococcal surface proteins are considered as alternative vaccine candidates because of their serotype-independence. Thus far, pneumococcal surface adhesin A (PsaA) has proven to be highly protective against colonization in animal models. Moreover, pneumococcal surface protein A (PspA) and pneumolysin have shown to elicit protection against invasive diseases. Future research will elucidate their true potential in protecting humans. In this paper we discuss the present knowledge on pneumococcal vaccines and the current status of novel vaccine strategies.

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### 1. Introduction

*Streptococcus pneumoniae* is a major cause of invasive diseases such as meningitis, septicaemia and pneumonia. Approximately, 1 million children under 5 years of age die of pneumococcal disease annually [1]. In countries where the incidence of *Neisseria meningitidis* and *Haemophilus influenzae* infections has drastically decreased through the introduction of vaccines against meningococci group C and *H. influenzae* type B, *S. pneumoniae* has become the major cause of meningitis and septicemia in children. In addition, the morbidity by *S. pneumoniae* through respiratory tract infections such as otitis media and sinusitis is enormous. Thirty to 50% of all patients with otitis media and a substantial percentage of cases of sinusitis and pneumonia are caused by pneumococci. Risk groups for serious pneumococcal disease include children under the age of 2 years, elderly and patients with immunodeficiencies [2].

Nasopharyngeal colonization by *S. pneumoniae* is common: probably all humans are colonized with this organism at least once early in life. Especially in circumstances of crowding, as in day-care centers, nursing homes, hospitals and jails, the risk of colonization with pneumococci is high [3–5]. Colonization is not usually followed by disease, since this is prevented by the innate and adaptive immune system. However, disturbance of homeostasis between host and pathogen, for example through viral infections, malnutrition or local damage of the mucosa, is associated with the development of (invasive) diseases [6–8].

Since the discovery of the antibacterial properties of penicillin by Fleming in 1929, many antibiotics have been used for treatment of pneumococcal infections. Recently, antibiotic resistance has become a worldwide problem, which limits the choice of antimicrobial agents. Therefore, prevention of pneumococcal disease has become of great interest. Up till now, many research groups have focused on the development of new effective vaccines to be used in particular risk groups including immunocompromised patients and children. In this article, we will discuss the history of pneumo-

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coccal vaccination, and we will evaluate the different vaccine strategies, which are currently undertaken. Furthermore, we will discuss the recent developments in novel vaccine strategies.

## 2. History

Sternberg and Pasteur were the first to identify *S. pneumoniae*, initially described as the pneumococcus [9]. Effective therapy against pneumococcal disease was reported only a few years later by Klemperer and Klemperer [10], who discovered the protective potential of patient serum against homologous organisms. The first preventive strategy was introduced by Sir Almroth E. Wright in 1911, who suggested that inoculation of killed, whole pneumococci might induce a protective effect against pneumococcal infections [11]. Unfortunately, this pioneering vaccine failed because only one of the two serotypes discovered that time was included, and the maximum applicable vaccine dosage was insufficient because of the relatively large inocula required. In 1926, Felton and Bailey [12] for the first time isolated pneumococcal capsular polysaccharides. This directly led to the first capsular PS vaccine, which proved its effectiveness by successfully aborting an outbreak of pneumonia at a state hospital in Worcester, MA in 1931 [13]. Because of the subsequent development of successful antibiotic therapy that could more effectively deal with pneumococcal disease, the vaccine's popularity decreased and finally the PS vaccine was withdrawn from the market [14,15]. However, despite the development of new classes of antibiotics, morbidity and mortality stopped declining [16]. An additional problem was the rapidly emerging penicillin and multidrug resistance. The first reports on resistance to penicillin were reported in the 1930s in Australia and New Guinea [17]. It took until 1977 before highly resistant pneumococci were reported in South Africa (MIC >1.0 mg/l). In addition, these strains were also resistant to other penicillins and cephalosporins [18]. In the years following, penicillin resistant pneumococci rapidly spread throughout the world. The cause of this international spread was mostly due to a few multidrug-resistant clones with serotypes 6B, 9V, 14, 19A, 19F and 23F [19]. These problems have led to renewed interest in vaccine strategies, resulting in 1977 in the production of a 14-valent pneumococcal PS vaccine by Robert Austrian and coworkers [16]. This vaccine was expanded in 1983 to a 23-valent vaccine and had a theoretical coverage of  $\geq 80\%$  of the pneumococci causing infections in adults. Unfortunately, the vaccine was less immunogenic in small children and immunocompromised patients [20]. This finding has accelerated the current search for new and promising vaccine candidates. In order to gain insight into the mechanisms of vaccine response we will first discuss the natural host defense mechanisms against pneumococcal disease.

## 3. Host defense against pneumococcal disease

The pneumococcal outer surface consists of a cell wall covered by a polysaccharide capsule. Capsule polysaccharides are highly heterogeneous and, thus far, almost 100 different capsular serotypes have been described. The polysaccharide capsule is the most important virulence factor of the pneumococcus as it protects the bacteria from phagocytosis. Capsular polysaccharides are highly immunogenic and antibodies against these polysaccharides protect against infection with the homologous serotype. The antigenicity of the capsule is type-specific, but cross-reactions occur because of shared polysaccharides [21]. The next layer, the cell wall, consists of polysaccharides, teichoic acid and several cell wall associated surface proteins. The cell wall is responsible for the intense inflammatory reaction that accompanies pneumococcal infection since it stimulates the influx of inflammatory cells. In addition, it activates the alternative complement cascade and induces cytokine production [21]. The cell-surface associated proteins are believed to specifically contribute to virulence as well. The most immunogenic part of the cell wall is the phosphocholine part of the teichoic acid, which is also playing a major role in the inflammatory process. The cell wall is shielded from the host response by the capsule which is completely covering it.

Pneumococcal clearance from the lung mainly results from phagocytosis and intracellular killing of the bacteria by neutrophils and alveolar macrophages. This process can only occur in the presence of type-specific immunoglobulins (IgG1 and IgG2, IgM and IgA) and active complement. This antibody-initiated complement-dependent opsonization, which activates the classical complement pathway, is believed to be the major immune mechanism protecting the host against infections with pneumococci. Pneumococci may escape this mechanism in the absence of serotype-specific antibodies, and consequently, may enter the host through the interstitial tissue of the lung resulting in lymphatic spread and subsequent blood stream invasion causing bacteremia [22].

The mechanism of clearance from the blood appears to depend on the interaction of type-specific antibodies (IgG) complement and phagocytic cells in the liver and spleen. The absence of the spleen or cirrhosis of the liver predisposes for severe pneumococcal infection. Congenital deficiencies in immunoglobulin or complement are also associated with predisposition to pneumococcal infection [21].

Non-capsular antibodies, for example immunoglobulins directed against cell wall components, may play a role in the host response to pneumococcal infection as well. Although most cell wall components are protected from opsonization and phagocytosis by the capsule, certain proteins may penetrate the capsule and may therefore be recognized by the immune system. So far, several animal studies have shown a protective effect of immunoglobulins directed against selected cell surface-associated proteins including pneumococcal surface protein A (PspA), pneumococcal

surface adhesin A (PsaA), and pneumolysin [22]. This T cell dependent immune response (TD), which is present from birth, induces mainly IgG1 subclass immunoglobulins. Type-specific immunoglobulins may be more effective if from the IgG2 subclass. The adult antibody response against capsule polysaccharides mainly generates these IgG2 subclass antibodies. However, the antibody response to (pneumococcal) polysaccharides is relatively immature in young children and generates predominantly IgG1 antibodies, which may result in an enhanced susceptibility to pneumococcal infections [23]. Thus, with the type of vaccination, the type of immune response may differ, and so may the level of protection. One has to take this into account when evaluating different (future) preventive strategies.

#### 4. Polysaccharide vaccines

At present, one pneumococcal PS vaccine is still licensed and marketed, i.e. Pneumovax 23 produced by Merck Research Laboratories, USA. These vaccines contain 23 purified capsular polysaccharide antigens (serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F) accounting for 85–90% of pneumococcal diseases [20]. These vaccines are effective in adults and children above 2 years of age. Several studies have also shown a protective effect in risk groups such as patients with sickle cell disease or asplenia, and elderly with underlying conditions including chronic obstructive pulmonary disease [20,21,24]. Unfortunately, children under 2 years of age, who suffer the highest rates of pneumococcal carriage and disease, and immunocompromised patients show a severely impaired antibody response upon this vaccination [25–28]. Furthermore, the PS vaccine is not effective against acute otitis media caused by *S. pneumoniae* [29]. In addition, the PS vaccines do not induce a T cell-dependent immune response. This implicates the absence of memory B cells and limits the period of protection. Finally, several capsule polysaccharides are poorly immunogenic. These include several serotypes associated with penicillin resistance [30]. These factors have led to the development of new pneumococcal vaccines, although this has not yet resulted in the complete replacement of the PS vaccines. The PS vaccines do have an efficacy of 61–75% in children over 2 years of age as well as in adults, and, thus, are still useful for various purposes [31,32]. Therefore, the Advisory Committee on Immunization Practices (ACIP) recommends this vaccine in (a) persons aged  $\geq 65$  years, (b) immunocompetent children  $\geq 5$  years who are at increased risk for illness and death associated with pneumococcal disease because of chronic illness, (c) persons aged  $\geq 5$  years with functional or anatomic asplenia, and (d) immunocompromised patients aged  $\geq 5$  years who are at high risk for infection. They also recommend vaccination for people living in high-risk environments with emphasis on American Indians and Alaska natives [20,33].

Several studies have shown an additional effect of the PS vaccine when used as a booster after a primary conjugate vaccination [34,35]. For example, Breukels et al. [36] showed a poor IgG2 response in otitis prone children when vaccinated with a conjugate vaccine only, but boosting with a PS vaccine could overcome this problem. It has been suggested that administration of conjugate vaccines in infancy followed by the administration of a PS vaccine after the age of 10 years would offer the prospect of reducing the total burden of pneumococcal morbidity and mortality throughout life [9,37]. At this moment, the ACIP recommends to use the PS vaccine as a booster 2 months after conjugate vaccination in children 2–5 years of age [33].

An emerging risk group for pneumococcal infections is that of HIV positive patients. The pneumococcal attack rate is 40 times higher in these patients than in seronegative individuals [38,39]. Therefore, the use of the PS vaccine is considered to be an important preventive strategy. However, due to a low peak concentration of protective Ig antibodies after vaccination, the protected period is significantly shorter ( $< 3$  years compared to 5 years in HIV negative adults) [19,40]. The impaired antibody response does not account for all serotypes. This is probably caused by the phenomenon that some antipolysaccharide responses are T cell dependent, which is impaired in HIV infected patients, while others are T cell independent (TI) [19]. Vaccination with conjugate vaccine, therefore, offers no solution either. This is because of the effectiveness of this vaccine, which depends mainly on the impaired T cell-dependent immune response in this patient group. So until new, more promising alternatives are found, the best strategy for this risk group appears to be revaccination with polysaccharides every 3 years. Recently though, in sub-Saharan Africa this strategy has proven to be inefficient. French et al. [41] did not only show a lack of efficacy of the 23-valent PS vaccine in HIV-1-infected Ugandan adults, but they also described evidence of deleterious vaccine-associated side-effects. Significantly more cases of pneumonia were seen in the vaccine recipients compared to controls. This phenomenon may be explained by transient stimulation of HIV-1 transcription after pneumococcal vaccination, but also destruction of polysaccharide-responsive B cell clones by pneumococcal polysaccharides has been suggested [41].

#### 5. Conjugate vaccines

Already in 1929, Avery and coworkers showed that covalent binding of capsular polysaccharides to proteins increases the immunogenicity of the polysaccharides [30]. This comprises linkage of capsular polysaccharides to a protein carrier, either by covalent binding or through reactive groups [30,42]. The proteins that have proven to be good immunocompetent carriers in the case of *H. influenzae* type b vaccines are tetanus toxoid, diphtheria toxoid and outer membrane protein from meningococcus group B [30,43].

Table 1

Vaccine efficacy by means of reduction in disease (invasive disease, pneumonia, RTI, otitis media), secondary outcome (ventilatory tube placement, antibiotic prescriptions), carriage, cross protection, replacement and resistance

Country <sup>a</sup>	Year	Number of children	Age in months	Vaccine schedule	Follow-up in months	Invasive pneumococcal diseases	Pneumonia (radiograph confirmed)	LRTI	URTI	Otitis media episodes	Ventilatory tubes	Antibiotic prescriptions	Carriage of vaccine serotypes	Cross protection	Replacement	Resistance colonizing strains
The Netherlands	2003	383	12–72	1/2 × 7-valent + PS booster	26					–			++ (<24 months) No (>24 months)	No	Yes No	
California	2002/ 2003	37868	2	3 × 7-valent + booster	24–42	+++	+			+	+	+				
Finland	2001	1662	2	3 × 7-valent + booster	18					+				Yes	Yes	
United Kingdom	2003	293	2	3 × 7-valent + PS booster	12–48								No		No	
Israel	2002	264	12–35	1 × 9-valent	24								++	6A yes 19A no	Yes	
Israel	2001	264	12–35	1 × 9-valent	22			+	+	+		+				
South Africa	1999	500	2	1 × 9-valent	9								++		Yes	++ <sup>b</sup>
Israel	1996	263	12–18	1/2 × 7-valent	3								++		No	+++ <sup>c</sup>

<sup>a</sup> Data are retrieved from Dutch OMAVAX trial [60,63]; California Kaiser Permanente trial [48,50–52]; Finnish otitis media study group [53,61,66]; Sheffield Institute for Vaccine studies, UK [54]; Soroka University Medical Center, Beer Sheva, Israel [55,56,58]; Pneumococcal Diseases Research Unit, Johannesburg, South Africa [64].

<sup>b</sup> Significant reduction in penicillin and cotrimoxazole resistant strains.

<sup>c</sup> Large reduction in all antibiotic resistant strains.

The difference in the immune response towards pure PS vaccines is the observed switch to a TD response promoted by the protein. This leads to the induction of memory B cells and an improved B cell response. In addition, it leads to a better immune response early in childhood. This is explained by the stepwise maturation of the human immune system with the earlier maturation of the TD response compared to antipolysaccharide antibodies (TI-2 response). Unfortunately, linkage of polysaccharides to proteins is restricted: too much carrier antigen may impair the antibody response to the polysaccharides by antigen competition or carrier-mediated epitope suppression [44,45]. Thus, protection is restricted to a certain number of serotypes. Conjugate vaccine variants with 4–11 serotypes linked to variable protein carriers have been designed, and all showed to be safe and immunogenic in infants and toddlers [35,46–48]. The most prevalent serotypes, correlated with invasive diseases and antibiotic resistance, were chosen as vaccine candidates. Thus far, the 7-valent pneumococcal conjugate vaccine Prevnar (Wyeth, USA) or Prevenar (Wyeth, Europe), containing polysaccharides of serotype 4, 6B, 9V, 14, 18C, 19F and 23F, has been approved by the Food and Drug Administration (USA), and the Committee on Proprietary Medicinal Products (Europe) for the prevention of invasive diseases in children. These vaccines have a potential coverage of over 85% of the pneumococcal isolates for the USA, 60–70% for Europe and around 55% for Asia [49]. Clinical efficacy of the 7-valent conjugate vaccine against invasive diseases has been convincingly shown by Black et al. [50–52] in the northern California Kaiser permanente trial (Table 1). Besides an efficacy of 97.4% against invasive diseases caused by vaccine serotypes, they also showed a significant impact on pneumonia and otitis media. This was supported by data of a Finnish vaccination trial [53]. Moreover, several investigators have shown a significant reduction in nasopharyngeal carriage of vaccine type pneumococci in infants as a result of different conjugate vaccinations [47,54–56]. Mucosal immunity in addition to systemic immunity would explain this decline in nasopharyngeal colonization. This is supported by the results of a second Finnish study, showing that a tetravalent pneumococcal conjugate vaccine induces both mucosal and systemic antibody responses in toddlers [57]. It was suggested that diminished nasopharyngeal carriage could lead to reduced person to person spread of the pneumococcal serotypes most often associated with disease and antibiotic resistance, and even to herd immunity [47,58,59]. Because of these promising data, the ACIP recommended pneumococcal conjugate vaccination for all children under the age of 24 months, and children 24–59 months of age when at risk of pneumococcal disease [20,33]. Unfortunately, the Dutch OMAVAX study investigating the efficacy of the 7-valent conjugate vaccine against recurrent otitis media in children 12 months to 7 years of age, showed no effect on the number of otitis media events despite good antibody responses [60]. The authors suggested that interference in an existing balance between host and pathogen by means of

vaccination may induce pneumococcal strain replacement. The appearance of a new pathogen, in turn, increases the risk of acute otitis media [61,62]. This might explain the different results of the two landmark studies in California and Finland investigating the effect of conjugate vaccination on otitis media in infants: at infant age the conjugate vaccination may prohibit or delay nasopharyngeal acquisition of the most prevalent pneumococcal serotypes, thus preventing or delaying early pneumococcal AOM until a later age. Replacement is therefore not possible in young children whereas the OMAVAX colonization data showed full replacement of vaccine type pneumococci by non-vaccine type strains [63]. With ongoing follow-up, more studies have reported this phenomenon [53,55,64]. The effect of replacement to the burden of invasive diseases still remains unknown, although one conjugate vaccination trial in the US recently reported 25% increase in invasive diseases caused by non-vaccine serotypes. Though this was not yet statistically significant, in the future it may become clinically relevant [65].

Meanwhile, the conjugate vaccine has been implemented nation wide in the United States [66]. For the European union, only Austria is currently (January 2003) recommending country-wide childhood vaccination [67]. However, most other countries, including Belgium, The Netherlands, Denmark, Finland, France, Germany, Ireland, Italy, Spain and the UK limit vaccine application to high-risk groups including patients with functional asplenia, immunodeficiencies, diabetes and chronic diseases like kidney, liver, lung, and heart diseases and children with cochlear implants [67]. The Achilles heel as presented by the Finnish national expert group appears to be the less favorable cost-effectiveness of the recommended four-dose vaccination schedule [66].

## 6. Immunogenic proteins

Though conjugate vaccines are highly protective against pneumococcal diseases, long-term vaccine failure due to the limited serotype coverage, and consequently, induction of replacement disease is realistic. Moreover, the vaccine is rather expensive, prohibiting implementation in most developing countries. [53,55,60,64,65,68,69]. In the last decade, several groups have investigated the use of pneumococcal proteins as potential vaccine candidates and promising results have been reported. Although many proteins including pneumolysin, pneumococcal surface protein A, pneumococcal surface adhesin A, choline binding protein A (CbpA), neuraminidase, and autolysin have been suggested as potential candidates, the proteins PspA, PsaA and pneumolysin are currently the leading vaccine candidates (Table 2) [70].

Pneumococcal surface protein A is a member of a family of structurally related choline-binding surface proteins, which are able to interfere with complement fixation. They block the initiation of the alternative pathway through reduction of the amount of C3b deposited on the

Table 2  
Evidence for the protective role of the vaccine candidate proteins PsaA, PspA and Pneumolysin against colonization, sepsis, pneumonia and otitis media

	Colonization			Sepsis			Pneumonia			Otitis media		
	PsaA	PspA	Ply	PsaA	PspA	Ply	PsaA	PspA	Ply	PsaA	PspA	Ply
Animal model*												
-knock out mutagenesis			+++ [92]		++ [94]	++ [94]				+++ [92]		
-monoclonal antibodies	++ [87]					+++ [93]						
-animal antibodies					+++ [82,117]							
-human antibodies	+ / +++ [87]				+++ [115]	++ [118]						
-active immunization	++ / +++ [87]	+ [78] ++ [82]	- [83]	+ [83,88] +++ [90]	++ [83] ++ [81]	++ [83]	+++ [90]	+ [83]	+ [83]		+++ [116]	
Human												
-immunogenic (+ or -):												
serum IgG	+ [112]	+ [112]	+ [112]							+ [113]	+ [112]	+ [110]
salivary IgA	+ [114]	+ [114]	+ [114]							+ [114]	+ [114]	+ [114]
-protective role		+ [115]								+ [113]		

(+): partial (protective) effect (< 50%); (++) protective effect of 50–90%; and (+++) 90–100% protection.

pneumococci. These proteins reduce the effectiveness of the complement receptor mediated pathways of clearance [71–74]. Although PspA shows considerable antigenic heterogeneity between different strains, Nabors and co-workers [70,76] found broadly cross-reactive antibodies to heterologous PspA molecules after immunization with a single recombinant PspA preparation [75]. This is supported by findings of Kolberg et al. [77] who showed that a combination of two monoclonals identify 94% of clinical isolates. Moreover, active immunization with PspA in animal models has proven protective against invasive infections and nasopharyngeal carriage [78–82]. In addition, Nabors and coworkers have performed the first phase I vaccination trial with a single recombinant PspA variant in humans. This vaccine showed to elicit broadly cross-reactive antibodies to heterologous PspA molecules [76]. Furthermore, these antibodies were found to protect mice challenged intraperitoneally with pneumococci [83,84].

Another important candidate is pneumococcal surface adhesin A (PsaA), a member of the family of metal binding lipoproteins. This protein is part of an ABC transporter complex thought to be involved in the transport of manganese into pneumococci [85,86]. The first immunization studies with PsaA showed significant protection against colonization but little to modest protection against invasive infections [70,87–89]. Recently though, Seo et al. [90] showed that oral vaccination with PsaA encapsulated in microalginate microspheres elicits significant protection against colonization, pneumonia as well as septicemia in mice. Because PsaA and PspA have different functions in virulence, an additive role for these proteins in vaccination was suggested. Indeed, promising results have been found for the combination of PsaA and PspA in prevention of colonization and otitis media in animal models [79,85].

Pneumolysin, also containing a choline-binding domain, is suggested to interfere with host immunity and inflammatory responses by a variety of functions, including complement fixation and inhibition of phagocyte function. It also inhibits ciliary activity in the bronchus and is therefore important in pathogenesis of pulmonary infection [3,91]. Knock-out mutagenesis of pneumolysin has proven its role in virulence in case of colonization as well as infection [92–94]. Several investigators have described the protective properties of pneumolysin against challenge with pneumococci in mice, be it against invasive diseases only [95,96]. PspA has shown complementary protection to invasive diseases in animals when used in combination with pneumolysin [70,79].

Other pneumococcal proteins that have shown potential as vaccine candidates are PspC (CbpA), the Pht family, putative proteinase maturation protein A (PpmA), autolysin and neuraminidase. PspC either contains a choline-binding domain like PspA and pneumolysin or a LPXTG motif like other gram-positive bacteria [97]. This protein is supposed to bind secretory IgA and to interact with human epithelial and endothelial cells [97,98]. Vaccination with PspC

has shown to be protective against sepsis in mice. Moreover, antibodies directed against this protein have shown cross-reactivity against PspA [99]. It is not yet clear though whether vaccination with PspC elicits protection against heterologous PspC type strains. The Pht family is one of cell surface-exposed homologous proteins representing histidine triad motifs of which several members have shown to elicit protection against different pneumococcal serotypes in a mouse sepsis model [100]. Importantly, these proteins appear to be highly conserved among pneumococcal strains, implicating a potential broad coverage. The putative proteinase maturation protein A is a highly conserved surface-associated protein which shows homology to members of the family of peptidyl–prolyl *cis/trans* isomerases. Mutagenesis of PpmA reduced virulence of pneumococci in a mouse pneumonia model. However, further *in vivo* studies are necessary to investigate the potential protective properties of this protein [101]. Pneumococci also produce two neuraminidases, NanA and NanB. These pneumococcal enzymes cleave *N*-acetylneuraminic acid from mucin, glycolipids, glycoproteins and oligosaccharides on host cell surfaces. Although the precise role in the pathogenesis of *S. pneumoniae* has not yet been identified, neuraminidase is presumed to contribute to adherence to mucosal surfaces by decreasing the viscosity of the mucus layer or through exposing cell surface receptors for pneumococci [102]. However, mutagenesis of neuraminidase A did not affect virulence of pneumococci after intraperitoneal challenge [103], which suggests a limited role in invasive disease. Pneumococcal autolysin (LytA) is, like PspA, CbpA and pneumolysin, a choline-binding protein, which contributes to virulence by mediating the release of pneumolysin and possibly inflammatory cell wall degradation products [94,104]. The exact role of autolysin in pathogenesis is still unclear, and controversial data have been reported regarding the protective properties of this protein [105].

So far, none of the proteins are considered to elicit species-wide pneumococcal protection. This can be explained by the occurrence of allelic variation within most individual proteins [75,97,105]. Antibodies raised against a single protein may not recognize allelic variants. Therefore, immunological interference using multiple variants of a single protein or using multiple proteins will limit immunological escape by the pneumococcus. Therefore, a combination of proteins should be considered in future protein vaccine strategies. Which combination of proteins should be chosen remains open for discussion. Although a combination of pneumolysin and PspA is suggested because of their protective effect against invasive diseases in animal models, the route of infection investigated in these models is either directly intravascular or via intraperitoneal challenge, and therefore not physiological. The natural route of infection with *S. pneumoniae* is believed to start with colonization, which progresses to (invasive) disease, by crossing of the natural physical and immunological barriers. Therefore, it seems rational to aim for prevention of colonization.

By preventing nasopharyngeal colonization of *S. pneumoniae*, horizontal spread of pneumococcal strains will be diminished as well, thereby enhancing herd-immunity. This theory supports the usage of PsaA as vaccine candidate as this protein has shown to elicit significant protection against pneumococcal colonization. In combination with PspA full protection may even become reality.

Several recent studies suggest that administrating the protein vaccine via the oral or nasal route is as effective as systemic application [78,81,82,90]. One of these routes is strongly preferable because of the high number of vaccines already administered intramuscularly or subcutaneously to children as part of community vaccination programs. Moreover, in contrast to pneumococcal conjugate vaccines and PS vaccines, protection is also expected in children with HIV/AIDS, even in a prolonged stadium, because of the intact mucosal immune response in these patients [90].

The addition of an adjuvant will be inevitable when full protection is pursued; recent studies have all shown a significant increase in protection when adjuvants are used [81,82,85,88–90]. Which adjuvant elicits the best protection should be further investigated. Finally, encapsulating the proteins should be considered because this may elicit additional systemical protection due to prevention of degradation in the stomach [90].

Care should be taken that the vaccine elicits a high level of cross-reactivity against the heterogeneous spectrum of subtypes to avoid escape by recombination events. Several of the potential proteins display complex mosaic structure as a result of horizontal (inter-species) exchange of gene parts [97,105,106,107]. However, for the PspA molecules, cross-reactivity between the different variants is present because they share cross-reactive epitopes [76,77].

On the other hand, cross-reactivity with species other than *S. pneumoniae* should be closely monitored; for example, Jado et al. [108] have shown close homology between the PsaA molecules of *S. pneumoniae* and PsaA molecules in three different viridans streptococci. The eradication of these species is undesirable because they may protect humans from respiratory tract infections by other streptococci and species [109–111]. All in all, thorough investigation of these proteins still is necessary before large-scale immunization studies in humans will become within reach.

## 7. Conclusion

At the moment, prevention of pneumococcal diseases is possible through 23-valent PS vaccines and a 7-valent conjugate vaccine. Although the PS vaccine has several disadvantages, of which the most important one is its minimal efficacy in children <2 years and immunodeficient patients, they are still useful for immunocompetent individuals  $\geq 5$  years of age who are at risk of pneumococcal infections [20,33]. The more recently approved conjugate vaccine, which contains the capsule polysaccharides of the seven

most prevalent serotypes conjugated to a carrier protein, is highly immunogenic in children <2 years of age, showing better immune responses and immunological memory. Unfortunately, the efficacy is limited due to the restricted number of serotypes included, and in the long run the efficacy will be threatened by serotype replacement. Therefore, pneumococcal proteins have been studied thoroughly to evaluate their possible role in future pneumococcal vaccination. First of all, it is suggested that these proteins can be used as carrier protein in a conjugate vaccine, eliciting protection against the remaining serotypes thus preventing serotype replacement. In this respect, the results from Kroon et al. [26], showing significant protection in mice by a conjugate vaccine containing polysaccharides of serotype 18C conjugated to pneumolysin, look promising. Secondly, if the proteins turn out to elicit sufficient protection by themselves or in combination, a pure protein vaccine could also be optional. Because of the additive roles in virulence and protection of pneumolysin, PspA and most importantly PsaA, a combination of these proteins is most promising. A major advantage of this combination is serotype-independent protection. In addition, this concept will limit the costs due to the relatively simple production of these recombinant proteins [22]. Distribution of this vaccine to third world countries would become possible, thus reaching a broader target group than ever before. Importantly, if the vaccine can be administered via the oral or intranasal route a major risk group, HIV/AIDS patients, will also benefit of the protection elicited by the mucosal immune-system which is unimpaired in these patients [90]. On the other hand, escape mechanisms of the pathogen in response to the immunological pressure is not unlikely if full eradication cannot be achieved. Moreover, cross-responsiveness to commensal species is realistic.

The effect of vaccination on the bacterial dynamics depends on the kind of protection that is elicited. When vaccination leads to the prevention of pneumococcal carriage, the horizontal spread of pneumococcal strains will also be diminished. This may lead to herd immunity resulting in protection of unvaccinated individuals against pneumococcal diseases. In addition, the prevention of nasopharyngeal colonization indicates the presence of mucosal immunity, which may also reduce mucosal diseases caused by *S. pneumoniae*. On the other hand, this may also lead to herd susceptibility towards non-vaccine sero- or genotypes and even alternative pathogenic species, and hence, disease burden. In contrast, when aiming for the prevention of invasive diseases without disturbing the nasopharyngeal niche, the risk for replacement disease is minimal. Systemic antibodies against proteins involved in invasion but not adherence may accomplish this. Unfortunately, positive ‘side-effects’ such as prevention of mucosal disease and the induction of herd immunity will not occur with this strategy.

In conclusion, thorough investigation of the efficacy and safety of these vaccine candidates and monitoring of side-effects have to be performed before human use is optimized. Until then, the available vaccines can be used in the preven-

tion of invasive diseases in infants but close monitoring of escape mechanisms and vaccine failures remains necessary.

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