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Next Generation Pneumococcal Vaccines

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Abstract

Currently licensed pneumococcal vaccines are based on the generation of antibodies to the pneumococcal polysaccharide, of which there are more than 90 different types. While these vaccines are highly effective against the serotypes included, their high cost and limited serotype coverage limits their usefulness worldwide, particularly in low resources areas. Thus alternative or adjunctive options are being actively pursued. This review will present these various approaches, including variations of the polysaccharide-protein conjugate strategy, protein-based strategies, and an inactivated whole cell pneumococcal vaccine. The immunological basis for these different approaches is discussed as well.

Introduction

Streptococcus pneumoniae causes approximately 11% of mortality worldwide in children under five [1]. Pneumococcus causes invasive disease including sepsis, meningitis, and, most frequently, pneumonia; it is also a common cause of primarily mucosal diseases, such as acute otitis media (AOM) and sinusitis. Since the introduction of conjugated polysaccharide vaccines (PCV) over 10 years ago, countries that have implemented universal vaccination programs have seen significant reductions in rates of pediatric invasive pneumococcal disease (IPD) due to strains covered by these vaccines [2]. While the success of PCVs has been substantial, their high manufacturing complexity and costs limit their use in developing nations where morbidity and mortality from pneumococcal disease are highest. Additionally, there are over 90 identified pneumococcal serotypes and the regional distribution of predominant serotypes varies. Therefore, an affordable vaccine that confers broad, preferably serotype-independent protection from pneumococcal disease remains a major global health priority.

Current vaccines

The longest standing pneumococcal vaccine currently available is Pneumovax®23 (Merck), comprised of purified polysaccharides of 23 different serotypes. Pneumovax®23 is recommended for adults over the age of 65 and some high-risk patient populations, but its use in children is limited as polysaccharides are poorly immunogenic in infants and children

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under the age of 2 years. For the purposes of this review, further discussion of currently available vaccines will focus on conjugated polysaccharide vaccines.

A 7-valent PCV (PCV7, Prevnar®, originally from Wyeth, now Pfizer) composed of seven different pneumococcal polysaccharides conjugated to a protein carrier (CRM197™, a cross-reactive mutant of a diphtheria toxin) was licensed in the United States in 2000, and in many parts of Europe shortly thereafter. Early clinical trials of PCV7 demonstrated that immunized infants would generate robust serotype-specific antibody responses, with associated reductions in pneumococcal bacteremia. Phase IV surveillance studies confirmed decreased rates of IPD and nasopharyngeal carriage due to vaccine-type strains in the post-PCV7 era [2,3]. Furthermore, rates of vaccine-type IPD were found to decrease in non-vaccinated populations, namely infants too young to be immunized and adults over 50, following implementation of universal PCV7 immunization in the United States, suggesting that effective herd immunity is offered by this vaccination regimen. By CDC estimates over 2/3 of the total IPD reduction in the US can be attributed to herd immunity.

At the same time, there is growing evidence to suggest serotype replacement is occurring, with non-vaccine serotypes contributing more significantly to rates of carriage and disease than in the pre-PCV7 era [4,5]. While the possibility of serotype replacement had been predicted years before the introduction of PCV7 [6], it remained uncertain to what extent replacement serotypes would be capable of causing clinical disease. Some emerging serotypes are associated with increased resistance to antibiotics and higher propensity for invasive disease, especially serotype 19A. Additionally, the efficacy of PCV7 in preventing pneumonia or mucosal disease such as AOM is less pronounced than its ability to reduce bacteremic disease.

To address these issues, several expanded valency (so-called 2nd generation) PCVs have been licensed (13-valent Prevnar®, Pfizer; 10-valent with 3 different protein carriers Synflorix™, GlaxoSmithKline Biologicals), or are currently under investigation (15-valent PCV, Merck). The expanded serotype coverage of these vaccines may further reduce IPD rates in the US and Western Europe, but the complexity and cost of these vaccines will undoubtedly continue to rise.

Thus alternative vaccine approaches are being considered and will be discussed here. These include new polysaccharide conjugate technologies which are less costly to manufacture; vaccines based on non-capsular protein antigens that are well conserved amongst the >90 known pneumococcal serotypes; and an unencapsulated killed whole cell vaccine. First, we will briefly summarize the current understanding of the mechanisms of immunity to pneumococcal infections and carriage.

Immunity to pneumococcal disease and colonization

Anticapsular antibodies are sufficient to prevent invasive disease such as meningitis and sepsis. Measurements of serotype-specific antibody titers and, more recently, opsonophagocytic activities (OPA) against various serotypes have been the mainstay of assessing immunogenicity of PCV preparations. What is less clear, however, is whether these antibodies are *necessary* for protection against invasive disease or whether these represent the natural mechanism whereby unimmunized children become resistant to pneumococcal infection. The invasive pneumococcal disease incidence for all serogroups peaks around the first year of life and falls in approximately parallel fashion thereafter, with a 50% reduction in incidence by age 2 [7]. These data suggest that the natural mechanism of immunity is unlikely to be primarily dependent on the acquisition of immunity to each serogroup: a) the incidence of invasive disease as children age is similar for all serogroups and b) this incidence declines well before anticapsular antibodies can be readily detected in

the sera of children. These findings thus suggest that naturally acquired immunity to pneumococcus may not be primarily dependent on the development of anticapsular antibodies.

Given the well-established increased susceptibility of IgG-deficient or asplenic individuals to pneumococcal infection, it seems reasonable to assume that antibodies directed against noncapsular component may contribute to protection as well. Several pneumococcal surface proteins are immunogenic in infancy and antibodies against them can be elicited by mucosal colonization (carriage), otitis media, or invasive disease. Furthermore, many of these proteins have been shown to be protective against sepsis when used as immunogens in mice. Sera from humans who have been immunized with pneumococcal surface protein A (PspA) or naturally-occurring antibodies to phosphorylcholine confer passive immunity in murine pneumococcal sepsis models. Data from mouse studies suggest that, as a whole, species antigens may be less potent than capsular polysaccharides when used individually but are synergistic in combination, forming the basis for the view that a protein-based vaccine may require several antigens in order to be effective, as will be discussed below.

Because pneumococcal pathogenesis begins with colonization of the nasopharynx, many research efforts have also focused on elucidating the mechanisms of immunity to pneumococcal colonization, primarily based on murine studies. In mice acquired immunity to colonization following mucosal exposure to either live or killed unencapsulated pneumococci (whole cell antigen; WCA) [8], is antibody-independent and CD4⁺ T cell dependent. This immunity to colonization is maintained in mice that genetically lack antibodies, IFN γ , or IL-4, but mice treated with neutralizing anti-CD4 or genetically lacking the IL-17A receptor are not protected by these exposures. A likely effector cell was identified as IL-17A producing CD4⁺ T_H17 cell. [9]. The recent finding that patients with autosomal dominant hyper-IgE syndrome (Job's syndrome), who are susceptible to recurrent mucosal infections with *Staphylococcus aureus* but also *S. pneumoniae* and other organisms, have a genetic mutation that results in the inability to differentiate T_H17 cells suggests that T_H17 cell deficiency may indeed be associated with a predisposition to pneumococcal infections.

Mouse models of pneumococcal colonization and disease

It is important to remember that, with the exception of great apes, the only natural host of pneumococcus is the human. Thus, despite the wide use of mouse models in the scientific literature, some caution should be used when interpreting the results obtained from these studies, as they may not necessarily apply to the human. Mice are readily colonized by pneumococci of multiple types [10]; in some mice using specific pneumococcal strains, colonization can be shown to last several weeks, which may be viewed as representative of what is believed to occur in humans [11]. In contrast, mice are highly susceptible to invasive infection by only certain pneumococcal types, generally of the lower serotypes/serogroups. Furthermore, the number of pneumococcal strains that can cause invasive disease in any particular mouse model is even more limited. There is a wide range of inocula used in mouse studies, which may also have an important influence on the apparent efficacy (or lack thereof) of any potential vaccine candidate. The susceptibility to infection is often highly variable across different animal host genotypes and the route of infection (e.g. intraperitoneal (IP) or intravenous (IV)) rarely mimics the natural mode of infection in humans. While many studies report the use of the IP or IV route of infection, intranasal inoculation of selected strains under anesthesia subsequently can also lead to invasive disease, in a pattern that may mimic natural infection following aspiration in humans [12,13].

Novel Vaccine approaches

For the purposes of this review, we will distinguish three vaccine approaches that are being considered.

1. Adaptations or modifications of the conjugate vaccine approach

The effectiveness of a capsular polysaccharide conjugate strategy has been well established, but as noted above, there are drawbacks to this approach, such as the cost and complexity of manufacture as well as the geographic differences in serotype distribution. At least two alternatives are being considered. The international nonprofit organization PATH is collaborating separately with the Serum Institute of India Ltd. and the China National Biotech Group's Chengdu Institute of Biological Products to speed the development of pneumococcal conjugate vaccines that target serotypes prevalent in low-resource countries and would be affordable for the developing world, using novel techniques to reduce cost of generating conjugates. Another strategy, also proposed by PATH, would involve the development of a hybrid approach, whereby a conserved pneumococcal protein (see below) would be used as a carrier for a limited number of polysaccharides chosen to meet or exceed WHO/GAVI target product profile [14].

2. Protein-based serotype independent subunit vaccines

An alternative approach that has been the focus of decades of research relies on the use of broadly conserved protein combination vaccines. Initially, most of the considered candidates were surface-expressed, to optimize antibody-accessibility. Two well-studied examples of these include pneumococcal surface protein (Psp) PspA, and choline binding protein (Cbp) (also referred to as PspC) (see table 1). While both of these antigens have sequence-variability in their immunodominant regions, the humoral immune responses they elicit have been shown to be cross-reactive among strains expressing heterologous versions of these proteins in experimental human carriage studies. Recombinant PspA has been safely given to humans in a phase I clinical trial and found to be highly immunogenic. Sera from humans immunized in this study passively protected mice from pneumococcal sepsis. Other conserved and well-characterized virulence factors include pneumolysin, a cholesterol dependent cytolysin, and pneumococcal surface adhesin (Psa) PsaA. Various toxoid derivatives of pneumolysin have shown efficacy in preventing invasive disease in mice but effective vaccines tend to require more than one protein in these studies. PsaA, a manganese binding lipoprotein, has been shown to reduce carriage and delay invasive disease in animal models; again the effect of this immunogen is most clearly evident when combined with others.

Reverse vaccinology has helped to identify a panel of antigens that might be promising vaccine candidates from a pool of thousands. Based on the reasoning that antigens well displayed on the surface of the bacteria are most likely to interact with host immune cells and effectors such as antibodies, earliest pneumococcal genomic scans identified proteins encoded by genes containing sequence patterns targeting the protein for surface expression. Scanning the genome for open reading frames with secretion signal motifs [15] or hydrophobic leader sequences likely to encode cell wall attachment sites [16] investigators identified several proteins including the pneumococcal histidine triad (Pht) family of proteins which have been the focus of studies by GSK [17].

Transposon libraries containing hundreds to thousands of isogenic strains with inserted mutations, each associated with unique molecular tags, have also been used to identify potentially protective antigens. These mutant strain libraries were then screened in various animal models of infection to determine which mutants showed depressed growth or

attenuated virulence *in vivo*. This method has led to increased understanding of which families of proteins are critical during various phases of infection and has helped confirm or identify many virulence factors such as neuraminidase NanA, autolysin LytA, pilus family proteins, and choline-binding proteins. Many of these proteins have shown promise as vaccine candidates in animal models of sepsis and/or carriage. Another approach involves the use of full proteomic screens whereby protein libraries are evaluated for immunogenic targets. One advantage of these screens is the flexibility in identifying targets that seem to be associated with a desired immune effect or response. This approach has been used to identify antigens recognized by antibody from convalescent sera of patients recovering from IPD [18]. A recombinant protein vaccine containing two of the proteins identified through this work as well as PsaA was well tolerated in a phase I clinical trial [19].

An important issue that deserves mention is that the mechanisms whereby antibodies directed against proteins provide protection in animal models are varied or not yet clearly defined. Despite many efforts, it has been difficult to demonstrate effective OPA of antibodies directed against pneumococcal proteins. Functional activities of antibodies can be defined, but are of a different type than OPA: for example, antibodies to pneumolysin can be differentiated according to their ability to neutralize the effect of the toxin and vaccine-induced human antibodies to PspA have been shown to prevent PspA-mediated inhibition of complement C3 deposition [20]. For antibodies to many other proteins, precise mechanisms of action have yet to be defined. The lack of a functional assay for a combination vaccine represents a surmountable but potentially important regulatory hurdle for the further development of protein-based vaccines.

In a more recent effort to tie the anticapsular- and protein-based approaches together, GSK and PATH have entered a collaboration to study a vaccine combining the conjugated polysaccharides in the 10-valent conjugate with an as yet undisclosed protein component. This approach can be viewed as potentially enhancing the scope of protection by the inclusion of conserved proteins and offers the possibility of study in clinical trials vs. a conjugate alone with a non-inferiority endpoint.

Finally, using a proteomic approach, our group with Genocera Biosciences and PATH identified antigens that are recognized by T_H17 cells from murine or human hosts that have been exposed to pneumococcus. The targets identified by this work appear to be different from those selected by antibody-mediated approaches and protect animals from colonization in a CD4⁺ T cell and IL-17A dependent manner [21]. Work is ongoing to determine whether combinations of these proteins may provide protection from IPD; one could envision a protein subunit vaccine that contains a combination of proteins that provide antibody- and effector T-cell-mediated protection against invasive disease and colonization, or potentially a combination of proteins and conjugated polysaccharide, as described above.

3. Killed whole cell pneumococcal vaccine

Over a decade ago, our group began investigating the possibility of using a killed whole cell pneumococcal vaccine, where a large number of pneumococcal antigens would be presented at once. Such an approach is not novel, since published reports of numerous trials using this strategy date back as early as 1911. Accurately determining the efficacy of this approach in preventing pneumococcal infections or complications after influenza is difficult but a recent meta-analysis of this topic has suggested efficacy [22]. We made several departures from these original studies. First, we reasoned that the use of an unencapsulated strain may increase the immune response to noncapsular antigens. We also removed the main autolysin of pneumococcus (encoded by *lytA*) and substituted a nontoxic TLR4-activating pneumolysoid for pneumolysin. Work in mice, performed in collaboration with Dr. Luciana Leite's group at Instituto Butantan and PATH, has confirmed that immunization with killed

whole cells using a variety of routes (intranasal, oral, sublingual, transcutaneous, subcutaneous, and intramuscular) and adjuvants (enterotoxins, enterotoxin mutants or nontoxic subunits or TLR agonists by the mucosal route, and aluminum hydroxide by the parenteral route) provides robust and reproducible protection against nasopharyngeal colonization (via CD4+ T cells) and invasive disease (via antibodies) [23]. Most recently, a GMP-grade whole cell vaccine manufactured at Instituto Butantan and administered parenterally with aluminum hydroxide was shown to confer protection in mouse models of carriage or aspiration pneumonia/sepsis [24]. Potential advantages of this approach include the very low cost of production (Instituto Butantan estimates that a dose of vaccine could be produced for pennies, Luciana Leite and Isaias Raw, personal communication), the serotype-independent mechanisms of protection and the possibility of conferring comprehensive immunity to colonization and invasive disease. Following a pre-IND meeting with FDA in 2010, plans are underway for the completion of further preclinical studies and IND filing, in anticipation of Phase I trials in healthy adult volunteers that may begin by end of 2011.

Conclusions

The advent and initial success of conjugate pneumococcal vaccines in 2000 brought about the hopes of reducing, if not virtually eliminating, pneumococcus as a major scourge of humanity. Since then, we have learned much about the ability of anticapsular antibodies to significantly reduce the burden of pneumococcal diseases both directly and indirectly, via the establishment of herd immunity. At the same time, we have also been reminded of the abilities of pneumococcus to adapt and overcome immunological pressures, and thereby potentially reduce the overall impact of the vaccine strategy. The scientific investigations, which date back several decades, aiming at the discovery of novel approaches to provide immunity to this pathogen, remain an urgent priority, both for developing and developed countries.

The breadth of approaches and the advent of novel techniques that were described above provide much reason for optimism. Many candidates are at the late stage of preclinical development and some have entered clinical trials. It remains to be determined, of course, whether any of these approaches will prove to be successful and/or feasible as human vaccines. As noted above, because pneumococcus is (almost) exclusively a human pathogen, and the animal models used for its study are imperfect, a reasonable dose of skepticism regarding the efficacy of any vaccine based on rodent models is very appropriate. Furthermore, because, as they age, humans gradually develop potent immunity to this pathogen, any suggestion of efficacy will need to await careful clinical studies in either the youngest children or the elderly. At the present time, it is premature to state whether a comprehensive immunization strategy may be based on a larger number of conjugates, combinations of polysaccharide conjugates and proteins, proteins alone, or even the whole cell vaccine approach. But, at the very least, it seems clear that the clinical evaluation of any of these candidates will help address many remaining questions in pneumococcal pathogenesis and prevention.

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Table 1

Selected pneumococcal proteins with demonstrable efficacy as immunogens in animal models

Protein family	Examples of antigen	Evidence of protection in animal models versus		Selected references
		Invasive disease	Carriage	
Choline Binding Proteins	PspA	+	+	[25,26]
	CbpA (PspC)	+	+	[13]
	PcpA ^c	+	–	[27]
	LytA	+	NR	[28]
	LytB/C	+	NR	[15]
Toxins	Pneumolysin	+	–	[29]
Sortase and sortase- dependent proteins	SrtA	+	–	[30]
	RrgA/B/C	+	NR	[31,32]
	NanA	+	+	[33,34]
	PrtA	+	NR	[15]
ABC (ATP-binding cassette) Transporter Proteins	PiaA and PiuA	+	NR	[35,36]
	PsaA	+	+	[37,38]
	PotD	+	+	[39,40]
	SP 2108	–	+	[18,21]
	SP 0148	NR	+	[21]
Enzymatic proteins	ClpP	+	+	[41,42]
	StkP	+	NR	[18]
Histidine Triad Proteins	PhtA/B/D/E	+	+	[43]
Other	PcsB	+	NR	[18]
	PppA	+	+	[44,45]

abbreviations: PspA: pneumococcal surface protein A; CbpA: choline binding protein A (also referred to as PspC: pneumococcal surface protein C); PcpA: pneumococcal choline-binding protein A; LytA: autolysin- an amidase; Lyt B/C: endo-beta-N-acetylglucosaminidase and lysozyme, respectively; SrtA: sortase A; RrgA/B/C: pneumococcal pilus 1 subunits; NanA: neuraminidase A; PrtA: serine protease; PiaA and PiuA: substrate-binding lipoproteins of two separate iron uptake ABC transporters; PsaA: pneumococcal surface adhesin A (a substrate-binding lipoprotein of a manganese ABC transporter); PotD: substrate-binding protein of a polyamine ABC transporter; SP 2108: TIGR4 locus name for substrate-binding lipoprotein of a maltose/maltodextrin ABC transporter; SP 0148: TIGR4 locus name for substrate-binding lipoprotein of a methionine ABC transporter; ClpP: caseinolytic protease; StkP: serine/threonine protein kinase; PhtA/B/D/E: pneumococcal histidine triad proteins; PcsB: protein required for cell separation of group B streptococcus; PppA: pneumococcal protective protein A; NR: Not reported