Short-chain oligosaccharide protein conjugates as experimental pneumococcal vaccines

W.T.M. Jansen & Harm Snippe

Eijkman-Winkler Institute, Heidelberglaan 100, 3584CX Utrecht, The Netherlands

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Streptococcus pneumoniae remains a major cause of acute respiratory bacterial infections, leading to approximately 1 million childhood deaths each year\(^1\). Antibiotic resistance is a growing problem in the treatment of pneumococcal infections\(^2\). Despite the widespread use of antibiotics, the mortality and morbidity of pneumococcal disease remains high\(^3\). In addition, resistant pneumococci are increasingly observed\(^3\)\(^-\)\(^5\). Recently, a report describing vancomycin tolerant pneumococci has alarmed the scientific community\(^6\). The main risk groups for pneumococcal disease are infants below 2 yr and elderly above 60 yr\(^7\). Worldwide, approximately 1 million children die each year from pneumococcal pneumonia\(^8\). Therefore, renewed efforts have been undertaken to develop effective vaccines against pneumococci.

**Capsular polysaccharide**

Pneumococci can be subdivided into more than 90 different serotypes, based on their capsular polysaccharide (PS)\(^9\). Pneumococcal capsular types differ significantly in their virulence\(^10\). Of the 90 serotypes known today, only a limited number of 20 serotypes cause the majority (90%) of disease\(^10\). Serotypes most commonly isolated from patients include serotypes 1 and 14 from the blood, serotypes 6, 10 and 23 from the cerebrospinal fluid and serotypes 3, 19 and 23 from the middle ear fluid of infants\(^11\). Serotypes 6A, 6B, 14, 19F and 23F are the main paediatric serotypes\(^9\). These serotypes are poorly immunogenic, which might be the reason for their success as a pathogen in infants. The capsular PS are very long polymers of either linear or branched repeating units consisting of 2 (serotypes 3, 37) to 8 (serotype 17A) monosaccharides\(^10\).

Host protection against *Streptococcus pneumoniae* is mainly mediated by complement and antibody dependent phagocytosis. Capsular PS are the most important virulence factors of pneumococci\(^9\). Capsular PS provide resistance against complement mediated...
phagocytosis and shield the inner structures of the pneumococcus from the immune system. As a consequence, only antibodies against the capsular PS have been shown to fully protect humans against invasive pneumococcal disease. However, PS are so called thymus independent antigens, which means that they do not recruit a T helper cell response. As a consequence, the antibody response is mainly restricted to IgM and IgA, affinity maturation does not occur, and B memory cells are not produced.

23-valent PS vaccine

The capsular PS of the 23 most prevalent serotypes have been included in the licenced 23-valent PS vaccine. This vaccine provides protection against 73 per cent of the strains, and reduces the risk of systemic infection in the adult population to 83 per cent. The efficacy of the vaccine, however is debatable in groups at risk for pneumococcal infections. In the elderly, ineffective antibody responses after vaccination have been reported. However, a meta analysis of clinical PS vaccination studies including more than 65000 patients, did not show differences in vaccine induced antibody levels between adults and elderly. In the elderly, avidity of these antibodies is lower, which might correlate with an increased vulnerability to pneumococcal disease. For infants, the poor immunogenicity of the PS vaccine is beyond doubt and thereby is unsuitable.

Polysaccharide-protein conjugate vaccines

The conventional 23-valent PS vaccine provides insufficient protection in groups at risk for pneumococcal infection. To overcome the thymus independency of PS antigens, PS are conjugated to carrier proteins, according to the immunological concepts developed in the mid seventies. These conjugate vaccines are immunogenic in infants, but in general, antibody responses in adults are not improved as compared to the conventional 23-valent PS vaccine. Recently clinical phase III trials have been conducted for several conjugate vaccines. These vaccines include the serotypes 4, 6B, 9V, 14, 18C and 23F. As carrier proteins, either dipheria toxoid CRM197, meningococcus B outer membrane proteins or tetanus toxoid are used. Several of these conjugate vaccines have been licenced and seem effective against pneumococcal pneumoniae, but to a lesser extend to otitis media. It is therefore tempting to assume that the major obstacles in the prevention of pneumococcal infections have been overcome. Despite the clinical and commercial potential of these vaccines, a number of major problems can still be envisioned.

Protective capacities of these vaccines have not been fully established yet, since information about protective opsonic antibody levels in humans is still lacking. In addition, the immunogenicity varies among the serotypes. Especially serotype 6B may be a problematic serotype in currently tested conjugate vaccines. Furthermore, multivalent conjugate vaccines are very expensive and therefore not appropriate for vaccination programmes in the developing world. The most important problem is the number of serotypes that has to be included in the vaccine. Although a multivalent conjugate vaccine that comprises the 9 different most virulent serotypes would cover 80 to 90 per cent of the pneumococcal serotypes isolated worldwide from patients, it is to be expected that a shift in incidence towards non-vaccine serotypes may occur, or new serotypes may emerge. Since the 23-valent PS vaccine has proven to be safe, and the immunogenicity of each serotype seems not to be suppressed by the other serotypes, similar benefits can be expected from multivalent PS conjugate vaccines. Nevertheless, the efficacy and safety of the vaccine should be controlled when additional serotypes have to be incorporated in the vaccine. Immunogenicity may be hampered by antigenic competition, carrier suppression, unwanted cross reactions with host antigens may occur and finally, since capsular PS are indirectly linked to cell wall-PS, increasing the number or doses of serotypes in the vaccine, will result in a similar increase of cell wall-PS content, which may affect the safety of the vaccine.

Protein vaccines

Taken into account these obstacles towards a safe and effective, worldwide pneumococcal vaccine, other vaccine strategies should also be considered. To overcome the disadvantages of serotype-specific vaccines, a number of pneumococcal proteins are being evaluated as vaccine candidates. These proteins include pneumolysin, autolysin, pneumococcal surface protein A (PspA), and pneumococcal surface adhesin A (PsaA). Although most proteins induce a certain level of protection
against pneumococcal carriage and invasive disease in mice, their protective capacities in humans remain uncertain\(^31,32\).

**Synthetic oligosaccharides in pneumococcal vaccines**

Pneumococcal capsular PS epitopes can be presented by synthetic oligosaccharides (OS). The synthetic components can be conjugated to a carrier protein with conventional coupling chemistry, to obtain semi-synthetic conjugate vaccines. The preparation of synthetic saccharides that represent fragments of pneumococcal PS antigens is traditionally difficult. Nevertheless, the combination of organic synthesis and biosynthesis using glycosyl transferases has lead to the production of numerous synthetic saccharides for serotypes 1-4, 6A/B, 7F, 8, 9A/V, 14, 17F, 18C, 19A/F, 22F, 23F, 27 and 29\(^33\). These saccharides have been used as inhibitors to determine the epitope specificity of anti-PS antibodies. In addition, synthetic OS can be used to study, and block, pneumococcal adhesion to epithelial cells\(^34-38\). Finally, coupled to carrier proteins, a growing number of synthetic saccharides is evaluated as a conjugate vaccine in murine models. Within the vaccine department at the University Medical Center, Utrecht in collaboration with the Bijvoet Centre in Utrecht, several synthetic saccharides are under investigation. Synthetic 6B, 17F and 23F saccharides induced opsonic anti PS antibodies in mouse models and rabbit models\(^33,39-43\). For all serotypes tested so far, anti-PS responses could be induced by very small OS fragments comprising only 1 repeating unit or less of the corresponding PS (Table). The serotypes listed in the Table belong to the most common ones isolated from adults and infants. Immune responses against the OS were highly specific and interestingly, some of the OS-protein conjugate vaccines even induced higher levels of anti-PS antibodies than the conventional PS-protein conjugate counterpart\(^31\).

Although the vaccine potential of small synthetic OS is clear from studies in murine models, their potential as vaccine candidates in humans is still uncertain. It is known that epitope size on pneumococcal PS can vary between serotypes\(^42,52\), between species\(^42\) and with age\(^53\). In contrast to mice, humans may require longer saccharides for an optimal immune response, presumably because of the better presentation of conformational epitopes\(^53\). Again, this may vary per serotype since 6A and 6B\(^41\), but not 23F\(^42\) OS fragments were recognized by human vaccination antisera, indicating the presence of epitopes for human anti-PS antibodies on the former but not the latter OS fragments.

A potential drawback of minimal synthetic OS conjugate vaccines is the absence of displaying

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Carrier-protein</th>
<th>OS chain length(^a) inducing (protective) anti-PS responses</th>
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<tbody>
<tr>
<td></td>
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<td>mice</td>
<td>rabbits</td>
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<tr>
<td></td>
<td></td>
<td>Anti-PS response(^b)</td>
<td>protection(^c)</td>
</tr>
<tr>
<td>3</td>
<td>CRM 197</td>
<td>di, tri, tetra</td>
<td>di, tri, tetra</td>
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<tr>
<td>6A</td>
<td>KLH</td>
<td>tri, tetra</td>
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<tr>
<td>6B</td>
<td>KLH</td>
<td>di, tri, tetra</td>
<td>tetra</td>
</tr>
<tr>
<td>14</td>
<td>CRM 197</td>
<td>tetra, octa</td>
<td>ND</td>
</tr>
<tr>
<td>17F</td>
<td>KLH</td>
<td>di, tri</td>
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\(^a\), number of monosaccharides within 1 OS fragment, coupled to a carrier protein; \(^b\), as measured by ELISA using PS as coating antigen\(^41\); \(^c\), measured in a mouse challenge model\(^41\); \(^d\), passive protection of mice by anti OS-antibodies raised in rabbits\(^41\); ND, not done
conformational epitopes, which are present on the whole PS. On the other hand, the thymus dependency of an OS-conjugate increases with shortening of the OS chain length. Therefore, for PS containing conformational epitopes, an optimum OS length has to be defined, long enough to express conformational epitopes, yet short enough to induce an optimal Th cell response. To obtain OS that contain several repeating units of the PS, three methods can be applied: partial hydrolysis of the PS, chemical synthesis, or biosynthesis. Several OS have been successfully prepared by hydrolysis. A disadvantage of this method is that the production of these OS is less well controlled leading to less defined products (size and exact bound hydrolyzed) and the presence of impurities. Chemical synthesis of OS of several repeating units starts to become possible, but is still very complex, requiring a large number of synthesis steps. The combination of chemical synthesis and biosynthesis might be a promising alternative in the near future to produce large OS at sufficient amounts. Information about the biosynthetic pathways of the capsular PS of serotypes 3, 14 and 19F has become available. Several glycosyltransferases are identified and cloned and one of them has already been produced at a large scale. Even conjugation of certain saccharides to a carrier protein or peptide might be performed in part enzymatically in the future, since several enzymes have now been identified that can glycosylate specific amino acids residues. In addition to new strategies to synthesize saccharides, recently developed techniques such as Matrix-Associated Laser Desorption time of flight mass spectrometry (MALDI-tof) facilitate and improve the characterization of the product.

Our findings and the work of others on synthetic OS-protein conjugates encourage the exploration of synthetic OS conjugate vaccines as a pneumococcal vaccine. Nevertheless, to establish whether animals and humans can produce antibodies against minimal synthetic OS, is only the first step towards a synthetic pneumococcal vaccine. Next step is to define the most immunogenic, protective epitopes on PS and optimize the presentation of such epitopes to the immune system.

In conclusion, considering the large number of serotypes, the generation of new synthetic saccharides should be stimulated. Depending on the serotype, when needed and when possible, natural PS may then be replaced sequentially by their (bio)synthetic OS counterparts in future semi-synthetic pneumococcal vaccines.

References


30. Sorensen UB, Henrichsen J, Chen HC, Szu SC. Covalent linkage between the capsular polysaccharide and the cell wall peptidoglycan of Streptococcus pneumoniae revealed by immunochemical methods. Microb Pathog 1990; 8 : 325-34.


