Designing meningitis vaccines

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Abstract Conjugate polysaccharide vaccines are a recent intervention to combat the relative inability of young children to mount an effective immune response against encapsulated bacteria, especially Haemophilus influenzae (Hib), Neisseria meningitidis (Nm) and Streptococcus pneumoniae (Sp). These organisms cause the majority of community acquired septicaemia and meningitis in UK children. Their capsular polysaccharides, important virulence factors in evading phagocytosis, are poorly immunogenic in young children compared to adults. Conjugation, by covalent linking, of the polysaccharide to an immunogenic protein, has been demonstrated for each of these organisms to produce good antibody response to the polysaccharide. Conjugate Hib vaccines have proven effective in reducing Hib meningitis and invasive disease in the countries that have introduced them. Pneumococcal conjugate vaccines have proven effective in preventing invasive disease caused by serotypes contained in the vaccines. Efficacy studies are awaited for pneumococcal conjugate vaccines.

Introduction

Vaccinology has developed rapidly in the last century, in keeping with the confident steps taken in the fields of immunology and molecular biology. Two centuries after Jenner's description of cowpox inoculation protecting 8 year old James Phipps at subsequent smallpox exposure, we are faced with an ever-widening array of vaccines. This review will discuss polysaccharide conjugate vaccines, a recent class of successful vaccines. These vaccines have been developed to overcome the relative inability of the infant immune system to mount effective immune responses against organisms (encapsulated) with polysaccharide capsules. We will outline the molecular rationale for these vaccines, their impact and implications for future conjugate vaccines.

Conjugate polysaccharide vaccines

Bacterial capsular polysaccharides have a significant role in virulence by virtue of very low activation of the alternative complement pathway. This helps the organism escape complement-mediated damage and subsequent phagocytosis in the absence of specific antibody (Ab). Increased production of capsular polysaccharide has been associated with pneumococcal strains of higher virulence. Capsular carbohydrate antigens typically evoke a T cell independent immune response and do not result in high levels of high-affinity Ab, especially in young children. In young children, this immature response to polysaccharide antigens (Ag) is reflected in high rates of invasive infection due to Streptococcus pneumoniae (Sp), Neisseria meningitidis (Nm) and Haemophilus influenzae type b (Hib). Even in adults, memory is not induced, therefore no booster response is seen following further exposure to the polysaccharide Ag.

Covalent linking of capsular polysaccharide Ag to an immunogenic carrier protein (such as tetanus or diphtheria toxoid) (Fig 1) is thought to recruit T cell help, resulting in a T cell dependent immune response. In infants and young children, this has the potential to elicit high levels of specific high-affinity Ab (Table 1). Processed peptide from the carrier protein is presented in conjunction with the MHC II receptor of the antigen-presenting cell, enlisting T cell help. Subsequent T cell-B cell interaction results in B cell stimulation and differentiation into Ab-secreting cells, producing high-affinity Ab against the polysaccharide and carrier protein, predominantly IgG3. A large pre-existing population of T helper (T_h) cells that recognise the T cell epitope of the carrier protein is needed for a maximal response. Previous priming with either the protein-polysaccharide conjugate or the carrier protein alone is required. This helps explain the need for at least two doses.

Key points

Conjugation of bacterial capsular polysaccharides to immunogenic proteins significantly improves antibody response to the polysaccharide

Conjugate vaccines induce immunologic memory effectively

Hib vaccine introduction has dramatically reduced invasive disease, including meningitis and epiglottitis

Hib and pneumococcal conjugate vaccines reduce carriage, possibly aiding herd immunity

Overall, conjugate vaccines have proven effective in reducing polysaccharide meningitis and invasive disease.

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Haemophilus influenzae type b (Hib)

Developed in the 1980s, the Hib conjugate vaccine utilises the capsular polysaccharide polyribosyl-ribitol phosphate
(PRP), a critical virulence factor, conjugated to an immunogenic carrier protein. Before the introduction of routine Hib vaccination, more than 95% of *H. influenzae* invasive disease was due to encapsulated type b strains, while the vast majority of *H. influenzae* carriage was due to non-encapsulated, non-type b strains. Since introduction of the vaccine in the UK in 1993, rates of invasive Hib disease have declined in children age 0–4 years (Fig 2) from 27.2 per 100,000 in 1991 to 1.4 per 100,000 in 1997. Reductions in Hib meningitis, epiglottitis, bacteraemia and bone and joint sepsis have all been observed. Carriage is also decreased in recipients, the 'herd immunity' effect of the vaccine.

Hib vaccination has been introduced in many western countries, with dramatic reductions in rates of Hib meningitis and epiglottitis. In a randomised trial in The Gambia of over forty thousand infants, it resulted in a significant decrease in radiologically proven pneumonia in vaccinated children, as well as a major reduction in meningitis and other invasive Hib disease. Hib vaccination in the UK occurs at 2, 3 and 4 months (combined with DTP, OPV administered orally at the same visit). This schedule confers protection from invasive Hib infection to at least five years of age, with 98% vaccine effectiveness.

The success of the Hib vaccine has encouraged the development of conjugate vaccines for the other encapsulated bacteria that are major childhood pathogens: *Neisseria meningitidis* (Nm) and *Streptococcus pneumoniae* (Sp). However, unlike Hib, both these organisms have multiple serotypes that cause invasive disease, increasing the complexity and cost of vaccine design. Current technology allows up to 13 serotype-determining polysaccharide antigens (Ags) (13-valent) in a conjugate vaccine, but far fewer than the more than 90 identified Sp serotypes.

### Meningococcal conjugate vaccines

Invasive disease by Nm in the UK is caused predominantly by group B (60–65% of cases) and group C (35–40% of cases). Group C disease is more common in older children and adolescents than group B disease, which has highest incidence in infants and young children. It has proved difficult to find safe, immunogenic epitopes within the poorly immunogenic group B polysaccharide with its structural homology to polysialic acid in human tissue, including developing and adult brain. Other vaccine strategies utilising, among other candidate Ags, lipopolysaccharide and outer membrane proteins, are being explored as an answer to group B.

Meningococcal polysaccharide vaccines have been licensed for use for over twenty years, singly as group A or C, or in combinations of A, C, W135 and Y polysaccharides.
However, the poor immunogenicity of the group C polysaccharide in children less than two years old, and poor induction of immune memory, have made the development of conjugate vaccines a priority. Possible induction of immune tolerance with repeated doses of meningococcal polysaccharide vaccine has been cited as a reason against routine use of polysaccharide vaccines. Different conjugate vaccines containing Nm group C polysaccharide (monovalent), A/C bivalent and A/C/W135/Y quadrivalent (Fig 3) are all under development, some in phase II (safety and immunogenicity) trials. Published trials so far show Nm conjugate vaccines to be safe and immunogenic. In November 1999, the first group C conjugate Nm vaccine was introduced into the UK immunisation schedule for infants and a step-wise catch up programme is currently underway to immunise all children up to the age of school leaving (15–17 years), dependent upon vaccine supply.

**Pneumococcal conjugate vaccines**

Sp is the most common cause of acute otitis media (AOM) and bacterial pneumonia, and is a significant cause of bacteraemia and meningitis in the young, elderly and at-risk groups (including individuals with immune deficiencies, abnormal splenic function, diabetes mellitus, and chronic pulmonary or cardiac disease). At least 90 serotypes of Sp have been identified; just five serotypes (6, 9, 14, 19, and 23) accounted for 69–74% of Sp isolates in England and Wales between 1993 and 1995. Serotypes causing AOM
appear similar to other invasive strains\(^{16}\). Although the serotypes vary slightly, a similarly narrow range of serotypes causes the majority of invasive disease world-wide.

The currently licensed pneumococcal polysaccharide vaccines contain up to 23 serotypes. However, controversy continues regarding the efficacy of these vaccines, with randomised controlled trials showing less convincing evidence than case-control studies\(^{17-21}\). The rise in penicillin-resistant and multiply resistant pneumococci in the last decade has also spurred the development of an effective pneumococcal vaccine. Between 1993 and 1995 in England and Wales, 14.7% of Sp isolates were fully resistant to penicillin, and 3.7% fully resistant to cefotaxime\(^{15}\). The serotypes commonly exhibiting antibiotic resistance are largely confined to those associated with invasive disease, possibly increasing the attraction of a vaccine covering these serotypes.

Pneumococcal conjugate vaccines, containing combinations of up to 11 serotypes (11-valent), have been shown to be immunogenic and safe in phase II trials\(^{12,22,23}\). They also reduce carriage of vaccine related serotypes, although a compensatory increase in non-vaccine related serotypes has been reported\(^5\). A preliminary report from a large scale phase III trial of a 7-valent conjugate vaccine in 38,000 US infants claims 100% efficacy for the primary end-point of invasive disease due to vaccine-contained serotypes (with no cases in the conjugate group and 17 in the control vaccine group)\(^{25}\).

The effects of conjugate Sp vaccines on carriage of the bacteria has raised concerns that an effective conjugate vaccine, while preventing infection and carriage by vaccine serotypes, may, by ‘competitive exclusion’, allow other serotypes to increase as causes of carriage and invasive disease. These concerns may well be addressed by the phase III trials underway in Finland, USA, South Africa and The Gambia, using Sp AOM and/or invasive disease as primary endpoints.

### Carrier proteins

The choice of carrier proteins for conjugate vaccines involves a variety of considerations. Proteins already utilised in the routine immunisation schedule have the advantage of known safety profiles and familiar epitopes for recognition by T cells. The classic vaccine proteins of tetanus and diphtheria toxoids are prepared by chemical inactivation of purified toxin from bacterial culture. A genetically derived mutant of diphtheria toxin, CRM\(_{197}\), and an outer membrane protein complex particle extracted from Nm group B cells, OMPC, have also been utilised as protein carriers (Table 2). Chemical inactivation induces random modifications, making coupling of the protein with polysaccharide more difficult and less reproducible than the known sequence and properties of a mutant protein like CRM\(_{197}\).

**Possible immunologic interference:** Carrier protein choice is important to elicit a maximal immune response to the polysaccharide Ag. Consistent use of the same carrier proteins may lead to `carrier induced epitope suppression', where exposure to the carrier protein may expand the number of carrier specific B cells, and direct a conjugate vaccine away from the capsular polysaccharide specific B cells\(^{26,27}\).

**Hib conjugate variation:** The different Hib conjugate vaccines (Table 2) have different immunogenic properties, despite all being safe and effective. PRP-D was found to be less immunogenic than other conjugate preparations in young children below 18 months of age, although it did induce immunologic memory\(^{28}\). HibOC (PRP-CRM\(_{197}\)) and PRP-T were both found to be immunogenic, achieving protective levels of anti-PRP Ab after two or three doses, with memory induced (a rapid boost response seen with further exposure to PRP). Interestingly, PRP-OMPC achieved a rapid early rise in Ab, often after a single dose. However, the final levels achieved after two or three doses were lower than with HibOC or PRP-T\(^{29,30}\).

### Conclusion

Polysaccharide conjugate vaccines have proven highly successful in combating the immunological vulnerability children have to the encapsulated bacterium Hib, and show promise of the same for Sp and Nm in the next several years. They reduce both invasive disease and carriage of the organism. Polysaccharide conjugate vaccines against group B streptococci, the major bacterial pathogen in community acquired neonatal sepsis, have demonstrated immunogenicity and efficacy in animal studies, utilising the type III

| Protein conjugate | Derivation | Hib vaccine            |
|-------------------|------------|------------------------|
| Diphtheria toxoid PRP*-D | Formaldehyde inactivation of Corynebacterium diphtheriae culture derived toxin | ProHIBit (Connaught) |
| Tetanus toxoid PRP-T | Formaldehyde inactivation of Clostridium tetani culture derived toxin | ActHIB (Pasteur Merieux) |
| PRP-OMPC | Outer membrane protein complex particle extracted from Nm group B cells | PedVaxHIB (Merck) |
| CRM\(_{197}\)-HibOC | Genetically derived mutant of diphtheria toxin, CRM\(_{197}\) | HibTITER (Wyeth Lederle) |

*PRP: polyribose phosphate
Capsular polysaccharide\(^3\). Pathogens potentially amenable to conjugate vaccines directed against polysaccharide virulence factors also include enteric pathogens such as *Shigella*, *Vibrio* and *Salmonella*.

**Glossary of terms**

**Epitope** – the conformation of part of an antigen that is recognised and bound by T-cell receptors and Ab.

**Affinity** – the strength of a bond between an Ab and the epitope of an Ag.

**Avidity** – the strength of bonds between an Ag (with all its epitopes) and the binding Abs.

**Capsule** – the polysaccharide outer coating of some bacteria (including Sp, Nm and Hib), helps the organism evade phagocytosis.

**Immunogenicity** – the ability of a vaccine to induce specific antibody production in a vaccinated individual.

**Serological correlate of protection** – a known level of specific Ab that provides protection from disease due to that organism. Many organisms, e.g. Bordetella pertussis, have no correlate of protection, while others, e.g. hepatitis B and Hib, have reasonable correlates of protection.

**Efficacy** – the ability of a vaccine to protect vaccinated individuals from specific disease. Usually determined in phase III trials, performed in healthy groups that may not necessarily reflect the general population.

**Effectiveness** – the effect upon specific disease within a whole population once the vaccine is introduced.

**PRP – polyribosyl-ribitol phosphate**, a capsular polysaccharide and virulence factor of Hib, used as the polysaccharide Ag in the Hib vaccine.

**Conjugate vaccine** – a vaccine using a capsular polysaccharide Ag of a pathogen covalently bound to an immunogenic carrier protein.

**Phase II trials** – clinical trials to assess the safety and immunogenicity of a vaccine.

**Phase III trials** – clinical trials to assess the efficacy of a vaccine, in addition to safety.

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**Corneal opacity following Bell's palsy**

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Pablo Picasso’s *La Célestine* (1904) shows the Barcelonian procuress, Carlota Valdivia. The title refers to the notorious character of the same profession in a 15th-century Spanish play. The painting is one of the great works of Picasso’s ‘blue period’ (1901–4), during which he strongly emphasised blue to express the deprivation, physical discomfort and ill health of those whose company he kept.

The elderly woman is blind in the left eye owing to a corneal opacity. The left side of her mouth is lower and the left nasolabial fold less evident than on the right. This combination of physical signs suggests a previous left sided Bell’s palsy, which resulted in inadequate left lid closure, ipsilateral corneal infection and subsequent scarring.