

Glycoprotein conjugate vaccines

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Abstract

The polysaccharide capsule which surrounds bacterial species like *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Salmonella typhi*, is a potent virulence factor. It protects the bacterium from phagocytosis, but capsule specific antibodies plus complement binding to the capsule opsonise the organism for phagocytosis and elimination. Purified capsules elicit T-independent antibody responses without a memory function, and are often poorly immunogenic in infants where much of invasive *H. influenzae* type b (Hib) and pneumococcal infection is seen. Covalent linkage of the polysaccharide, or fractions thereof, to immunogenic carrier proteins creates glycoconjugates which are T-dependent antigens and which prime for boosting either with the glycoconjugate or the capsular polysaccharide; During the 1990s, four Hib glycoconjugate vaccines have been introduced and in countries that have vaccinated the majority of children, the success has been stunning. In countries with very high immunization coverage the disease has been virtually eliminated and, to a decline of over 95% in countries with slightly lower vaccine rates. Worldwide use of Hib glycoconjugate vaccines offers the possibility of elimination of invasive Hib disease. Pneumococcal (11 serotypes with coverage of approximately 85% of invasive disease) and meningococcal (A, C, W 135, Y but not B) glycoconjugates are in pre-registration phases and offer the prospect of being as successful as the Hib glycoconjugates. © 1999 Published by Elsevier Science Ltd. All rights reserved.

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1. Introduction

Several bacterial species are surrounded by a polysaccharide capsule made up of either a polymerized homopolymer, like $\alpha_2, 8$ or $\alpha_2, 9$ linked sialic acid residues in *Neisseria meningitidis* groups B and C, respectively, or polymerized repeating units made up of di- to pentasaccharides as in most *Streptococcus pneumoniae*. The capsular structure protects the bacterium against phagocytosis and is a potent virulence factor. Once inside poly-morphonuclear leucocytes the capsulated bacterium is rapidly killed. The host responds to the infection with production of specific antibodies which bind to the capsule thereby opsonising them for phagocytosis, a process mediated via both Fc and complement receptors. The antibody-dependent complement-mediated killing is thought to occur via a final insertion of the C8–C9 complex in the bacterial membrane.

The medical impact of infections caused by encapsu-

lated bacteria and in particular by pneumococci, was evident in the beginning of the century. The immunological properties of bacterial capsular polysaccharides became the target of several investigators in the 1920s and 1930s. In the mid-1940s it was evident that

1. Capsular polysaccharides, on killed cells or 'purified', elicited type-specific protective immune responses [1–4]
2. Infants and young children did not respond with type-specific antibodies neither after disease nor after vaccination [5–8]
3. Type-specific antibodies were the effector molecules which conferred protection [7,9]
4. Vaccination with polysaccharides reduced the carrier rate of bacteria of the same types as in the vaccine [4]
5. Glycoconjugates using haptenic saccharides covalently linked to a carrier protein, could in rabbits induce antibody responses which were high titered,

boostable and protected against challenge infection [10,11]

However, the successful introduction of antimicrobial agents put an effective stop to the further development of both capsular polysaccharide and glycoconjugate vaccines for a few decades. A few things rekindled the interest in vaccination in the 1970s. First it became increasingly evident that antimicrobials, although largely successful, was not the ultimate solution to handle infections. Treatment failures and the emergence of antimicrobial resistant strains forced a rethinking and the role of prophylactic immunization became one alternative. Advances in immunology with delineation of B and T cell responses, and the role of T cells for the immunological memory functions, as well as the structural elucidation of the capsular polysaccharide opened the way for development of defined glycoconjugates.

2. Haemophilus influenzae type b (Hib)

Haemophilus influenzae is a gram-negative coccobacillus which frequently colonizes the oropharynx of man. Most *H. influenzae* strains are non-encapsulated, (non-typable *H. influenzae* — NTHI), but a few are surrounded by a polysaccharide capsule. Six capsular types, a to f, have been recognized with type b being the most common and virulent. *H. influenzae* type b, Hib, causes meningitis, epiglottitis, septicemia, facial cellulitis, pneumonia, arthritis and a variety of less common forms, i.e. invasiveness and dissemination is a characteristic trait of Hib infection.

Most Hib infections in the prevaccine era occurred in children, less than 5 years of age. The rates of Hib disease varied, but invasive disease in Europe ranged from 30 to 60 cases per 100,000 children less than 5 years old [12]. In the US the corresponding figures ranged from 50 to 100 per 100,000 children. Some population groups have been reported to have much higher rates of disease; thus native Americans and Australian Aborigines have had rates up to 300 per 100,000. Invasive Hib infections are uncommon in adults unless there is predisposing underlying disease. An annual incidence of 0.22 per 100,000 has been reported [13].

The peak incidence of Hib disease in the prevaccine era was in children between the ages of 5 and 12 months, coinciding with the waning of maternal antibodies and prior to appearance of anti-capsular antibodies. A pivotal large scale double-blind study in Finland in 1974 comprising almost a 100,000 children between 3 months and 5 years of age showed that (i) children > 18 months were protected with an efficacy > 90%, (ii) children between 12 and 18 months had questionable protection, and (iii) no protection was

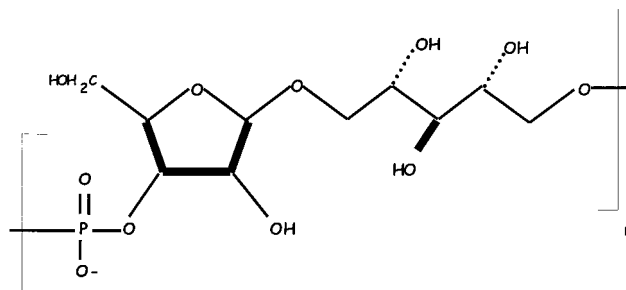


Fig. 1. *Haemophilus influenzae* type b repeating unit: structure of polyribosylribitolphosphate.

seen in the age group of 3 to 12 months [12,14]. This study certainly was a trigger point for the development of Hib glycoconjugate vaccines. After the pivotal studies of the 1920s to 1940s significant advances had been made (i) the role of T cells and B cells was being clarified, (ii) the structure of the PRP capsule (Fig. 1) was determined, and hence targets of chemical coupling strategies became evident (Fig. 2), and (iii) the immunological memory mechanisms were unraveled.

2.1. Conjugation strategies

The capsular polysaccharide of Hib does not contain chemically reactive groups such as amino or carboxyl moieties that can directly be covalently linked to a protein carrier (Fig. 1) was determined. However, the presence of both the ribitol and the phosphodiester in the repeating unit makes the polysaccharide susceptible to derivatisation. The periodate oxidation creates –CHO groups which by reductive amination are coupled to a carrier protein. This procedure was used by Anderson and coworkers at Praxis to create HbOC (Hib-Titer[™]) [15] Robbins et al. [16,17] used adipic acid dihydrazide (ADH), a bifunctional nucleophilic spacer, to facilitate binding of the capsular polysaccharide to the carrier. Cyanogen bromide is then used to introduce an active group into the ribitol moiety of

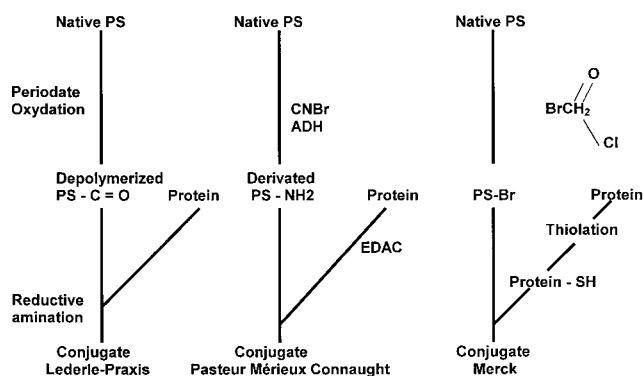


Fig. 2. Coupling schemes for the synthesis of *Haemophilus influenzae* type b glycoconjugates (15,16,17,18).

the repeating unit. The creation of a Hib adipic hydra-
zide derivative subsequently allows it to be conjugated
to tetanus toxoid by carbodiimide-mediated conden-
sation. The resulting conjugate (PRP-T, Act-Hib[™]) has
multipoint attachments between the polysaccharide
and the carrier protein and is referred to as a lattice
structure. A prior strategy first coupled ADH to the
carrier protein. This methodology was used by
Connaught for making the PRP-D (ProHibit[™]) con-
jugate which has been less immunogenic than PRP-T,
and is little used today.

A different conjugate, PRP-OMP, was developed by
scientists at Merck, Sharpe and Dohme [18]. They con-
jugated the PRP moiety to an outer membrane protein
complex from a *Neisseria meningitidis* groups B strain
(PedVax Hib[™]).

Thus there are three, currently used, different PRP
conjugates in the market with differences in (i) carrier
protein used (ii) linkage type with or without spacer,
and (iii) polysaccharide size, and linked at end(s) or
in the chains (Fig. 2).

The three vaccine types, not unexpectedly, differ in
their immunogenicity. HbOC and PRP-T behave in
the same manner each requiring two or three doses to
generate substantial antibody titers. PRP-OMP elicits
strong antibody responses already after a single dose,
and subsequent doses add little to the immune re-
sponse. Thus PRP-T and HbOC have been evaluated,
and are used, in three-dose primary immunization
series, whereas PRP-OMP can be used in two-dose pri-
mary series.

2.2. Correlates of protection after vaccination

The protective role of antibodies to the Hib capsular
polysaccharide was known already in 1933 [7]. The
inverse correlation of disease occurrence and the pre-
sence of bactericidal antibodies in serum of individuals
of different ages was convincingly demonstrated. The
pivotal Finnish study with purified PRP as vaccine
demonstrated the same relationship for type b specific
anticapsular antibodies [12].

Adults regularly have antibodies to PRP. Based on
the argument that Hib disease is rare in adults as a
consequence of 'protective antibodies' and that 95% of
adult sera analyzed had $>0.04 \mu\text{g/ml}$ of anti PRP anti-
bodies a limit of $>0.15 \mu\text{g/ml}$ was arbitrarily set as a
level for short-term protection [14,19]. In studies of the
post-immunization antibody concentrations seen in
vaccines in the Finnish PRP study a concentration
of $\geq 1.0 \mu\text{g/ml}$ predicted protection over the following
year [20]. Thus the concentrations $>0.15 \mu\text{g/ml}$ and
 $>1.0 \mu\text{g/ml}$ are used as correlates for short-term and
long-term protection, respectively. It is to be noted,
however, that these antibody concentrations are the
result of immunization with the T independent PRP

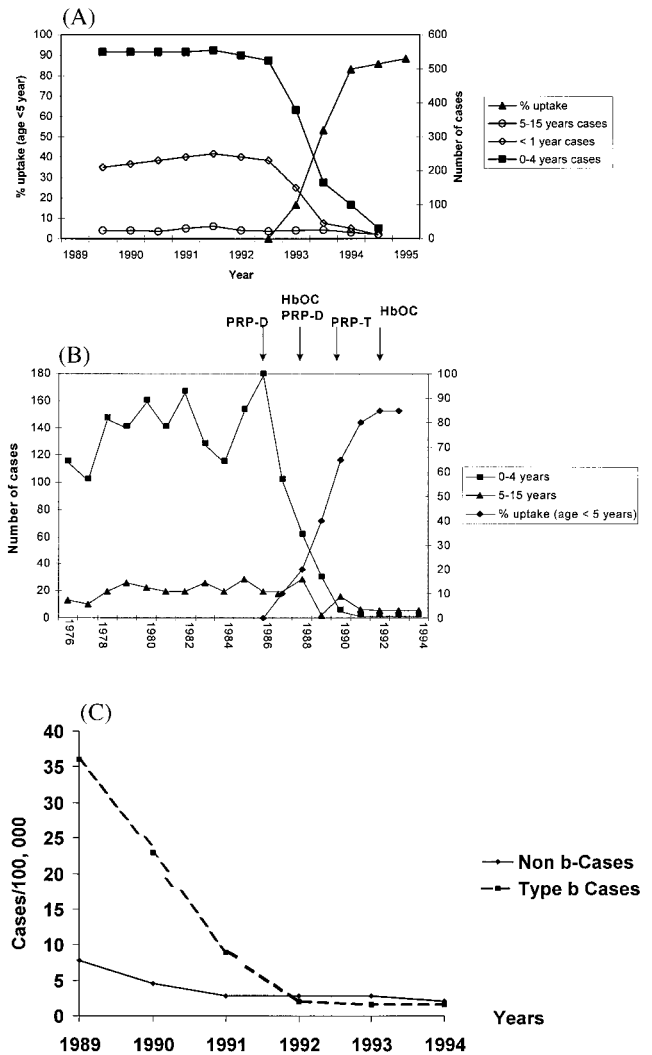


Fig. 3. (A) Number of cases of invasive *H. influenzae* type b disease in England and Wales. (B) Number of cases of invasive *H. influenzae* type b disease in children in Finland. (C) Incidence of invasive *H. influenzae* type b and non-b disease in the United States. From (80).

polysaccharide. Do these levels have any relevance for protection in an infant primed with a glycoconjugate for memory responses? It is probable that lower concentrations, with B cell clones which rapidly can expand upon stimulation, than those used as 'protective levels' following Hib conjugate vaccination are 'protective'. To determine such protective levels is, however, difficult. A better approach in the evaluation of glycoconjugate vaccine immunogenicity should be to incorporate a marker of priming. Memory responses are characterized by the production of high-avidity antibody, i.e. antibodies strongly binding to the antigen. Thus avidity can be considered as a surrogate of successful priming. In a recent study, it was shown that antibody avidity was relatively low following primary immunization, and significantly higher following boosting [21]. Most of the increase in avidity was seen

in the months after primary immunization. The concern with 'too low' anti-PRP titers after vaccination with combination vaccines containing acellular pertussis and Hib conjugate in the same formulation highlight a need for a better understanding of the relationship between primary antibody responses and long-term protection.

2.3. Hib glycoconjugate vaccine efficacy

The introduction of Hib conjugate vaccines has had a profound effect on the overall burden of Hib disease. There has been $\geq 97\%$ reduction in Finland, the UK and the USA, where different Hib conjugates have been used: in Finland PRP-D followed by HbOC [22], in the UK PRP-T [23] and in the US HbOC ($> 90\%$ up to 1994), Figure 2 in [24].

In Finland and in the US a primary series with three doses at 2, 4 and 6 months of age were used with a booster dose in the second year of life. In the UK the primary series also contained 3 doses given at 2, 3 and 4 months of age but with no booster [23]. The reported efficacy for infants 5–11 months of age was 99.1%, for 12–23 months old infants, 97.3% and for 24–35 months children it was 94.4%. Although this shows a gradual fall in the efficacy as children approach the age of 3 years, the 95% confidence limits for efficacy in each age group were found to overlap [23]. The estimated protection remained high at about 95%. The authors challenged the concept that a booster may be required in the second year of life to ensure that protection persists until school age (more than 95% of invasive Hib disease occurs before the age of 5). A basis for this proposal is a high vaccine coverage (probably $> 90\%$) with a resulting herd immunity. An added benefit of the routine use of Hib conjugate vaccines is a substantial reduction of oropharyngeal carriage of Hib [25,26]. The reduced colonization in immunized children most likely reduces transmission and hence children who lack protective antibodies have a reduced probability of being infected (see also Fig. 3).

The use of a carrier protein in the glycoconjugate vaccine may improve the immune response in yet another way. If the infant is primed by a prior tetanus toxoid vaccination the antibody response to PRP-T was found to be improved [27,28]. In the British study young infants (3–11 months of age) were found to have a high level of protection from 1 week after just one dose of vaccine [23].

2.4. Genetic basis for vaccine failure

Gm and *Km* genes code for the antigenic determinants of the heavy and light chains of immunoglobulins, respectively. *Gm* groups are localized to the

constant region of the γ heavy chain and thus are limited to IgG classes and each are exclusive to one of the four IgG subclasses. The *Gm* allotypes are located on chromosome 14. Many of the *Gm* factors are inherited together. The *Km* factors are present on χ light chains and therefore represented in all classes of immunoglobulins. The *Km* allotypes are found on chromosome 2.

There is significant variation in *Gm* and *Km* allotypes among different races in human populations [29], and important allelic associations with *Gm* and *Km* allotypes in the genetic predisposition to some infectious diseases [30,31]. The biological role of the polymorphisms of *Gm* and *Km* is not understood, but evidence suggests that the polymorphism is protective at the population level: lack of it is associated with higher levels of infectious disease [32] and is specifically associated with lower immune responses to certain infections [33].

The responsiveness of healthy white adults and children to Hib PRP is significantly associated with the *Km* (1)-negative allotype [34,35]. When the Hib glycoconjugate was used the association between the *Km* (1)-negative allotype and the PRP disappeared [36].

It appears that the responsiveness, or lack thereof, is specific to the particular type of polysaccharide. A woman unresponsive to PRP, responded readily to pneumococcal and meningococcal capsular polysaccharides as well as *H. influenzae* outer membrane proteins [37]. Immunization with PRP-T elicited only a minimal response, and not until multiple doses given at 0, 2, 4, 8 and 15 months, did she reach a concentration of 3.1 $\mu\text{g/ml}$. Thus a selective deficiency of antibody to PRP could be overcome by repeated injections with PRP-T, i.e. a need for an individual vaccination scheme beyond what is used in routine immunizations.

Response to PRP has also been linked to the *G2m*-positive allotype [23,38]. However, there is a lack of a persistent association across racial or ethnic groups regarding *Gm* and *Km*. Hence other factors must interact with the roles of the products of the *Gm* and *Km* allotypes. Although the use of glycoconjugate vaccines may overcome some of the allotype associations with the T-cell independent responses to polysaccharide vaccines, it may not be the panacea we hoped for.

3. Streptococcus pneumoniae

Streptococcus pneumoniae continues to be a major cause of morbidity and mortality in adults and children despite the availability of effective antimicrobial therapy. As for Hib the virulence is caused by a capsular polysaccharide, and there are now 90 different capsular types described [39]. Invasiveness is dependent on the capsular structure rather than on the amount being

produced. The polysaccharide is not toxic in itself, but inhibits phagocytosis.

The immunological defence against pneumococcal infection depends on type specific anticapsular polysaccharide antibody interacting with complement to opsonise the bacteria and have them prepared for phagocytosis and clearance. Defects in any of these components of the host defence, i.e. antibody production, complement factor deficiency, splenic dysfunction including splenectomized individuals, or neutropenia are usually associated with an increased risk of serious pneumococcal infection. Of the components type specific anticapsular antibodies are by far the most important. The importance of antibody to recovery from pneumococcal infection was shown already in the 1930s. The immunogenicity and immunochemistry of pneumococcal capsular polysaccharides has been recently reviewed [40,41].

Pneumococci causes a wide variety of infections ranging from relatively mild mucosal infections like acute otitis media, to more serious bronchopneumonia and potentially life threatening meningitis. Pneumococci colonize the respiratory mucosa of both healthy and sick individuals. The carrier rates are higher in children than in adults, even higher in children attending day care centers than those staying at their homes, and still higher in those with respiratory infections compared with healthy children [42,43,44].

Clinical illness is a result of the spread of the pneumococci to tissues from the oropharynx. Although carriage may result in infection it appears as if the period just after acquisition is a period of high risk [42]. Epidemiological studies in the 1980s have found an annual incidence of pneumococcal bacteremia to vary between 9 and 18 cases per 100,000 persons of all ages [41]. In children there were 105 and 234 cases per 100,000 individuals, white and black respectively. There appear to be racial differences with Blacks and Native Americans having rates approximately 2.5 times those of Whites and Hispanics. Even higher rates have been found among the native population of Alaska. It has been estimated that in the US there is on an annual basis 3000 cases of meningitis, 50,000 of bacteremia, 500,000 of pneumonia, and 7,000,000 cases of acute otitis media [41,45,46]. Approximately 40,000 deaths caused by pneumococci occur each year in the US and it has been estimated that approximately half are potentially preventable with pneumococcal vaccines.

In developing countries pneumococcal infections cause a significant disease burden. It has been estimated that acute lower respiratory infections caused by pneumococci account for more than 4 million deaths annually, most of them in children being <5 years of age [44].

Thus it is evident that a pneumococcal vaccine elicit-

ing protective immunity in the very young as well as the elderly would have a significant public health impact.

3.1. Capsular types

Of the 90 capsular types identified only a fraction are common causes of pneumococcal disease. The seven most commonly isolated serotypes cover up to 85% of all pneumococcal strains causing invasive infections in Finnish, Israeli and US children [47].

A nanovalent vaccine for global use including serotypes 1, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F would cover approximately 80% of strains causing infections in industrialized countries and approximately 70% of strains in developing countries [48,49].

There exists pneumococcal polysaccharide vaccines which are composed of the 23 most common pneumococcal serotypes. They represent 85–90% of the serotypes that cause invasive infections in adults in industrialized countries. As for Hib young children do respond poorly to most serotypes up to the age of 2 years, and the least immunogenic serotypes (6B, 14, 19F, 23F) up to the age of 5 to 10 years [49,50]. The polysaccharide as a T independent antigen also fails to induce a memory function.

The structure of each polysaccharide serotype differs from that of others serotypes and from that of PRP. Hence different coupling strategies have to be devised for each polysaccharide, either in its native or sized form (often incorrectly called oligosaccharide). The manufacturers are using the same overall strategies as for the Hib conjugates, i.e. reductive amination, ADH spacer and linkage to a *N. meningitidis* OMP complex. The carriers used are the same as for Hib conjugates, i.e. tetanus and diphtheria toxoids [17] the genetically toxoided diphtheria toxin CRM 197 [51] and the *N. meningitidis* OMP complex [18].

The resulting pneumococcal conjugate vaccine will contain from 9 to 11 different polysaccharides. However the amount of carrier protein should be restricted even if tetanus toxoid can prime for a higher antibody response for PRP-T [23], too much carrier antigen may impair the antibody response to the polysaccharides by antigen competition or carrier-mediated epitope suppression [52,53].

Several conjugates have been through the phase 1, 2 and 3 trials and several conclusions can already be drawn: (i) in adults the conjugates have been well tolerated although local reactions at the injection site appear to be more common than after vaccination with capsular polysaccharides [54], (ii) in toddlers all conjugates tested have been immunogenic, (iii) in infants all conjugates tested have been well tolerated and no immediate, serious vaccine related adverse events have been reported. Local reactions have been

similar to those seen after Hib conjugates. The reaction rates have been lower and their intensity less marked in young infants than in adults, (iv) there is priming for anamnestic IgG antibody responses to subsequent boosting with polysaccharide vaccine [55,56]. The highest dose (10 μ /ml of each polysaccharide) induced the strongest response after primary immunization (2, 4, 6 months of age), but the booster response was greatest in the group primed with the lowest dose (1 μ /ml of each polysaccharide), (v) a significant reduction in the carriage of vaccine related strains was observed after primary immunization suggesting that transmission of specific pneumococcal serotypes most often associated with disease at least partially be controlled by immunization [57], (vi) Wyeth-Lederle's heptavalent Pnc-CRM vaccine was 100% efficacious against invasive disease in a study in Northern California: 17 cases in the control group and 0 in the vaccine group (2, 4, 6 month primary series) (personal communication), and (vii) no antibody correlates for protection are yet available.

Available information suggest that we may have efficacious pneumococcal glycoconjugate vaccines on the market just after the turn of the century. Efficacy most likely will first be demonstrated for invasive infections (bacteremia and meningitis) to be followed by demonstrated efficacy against mucosal infections, i.e. acute otitis media.

3.2. Genetic regulation of immune responses

Healthy adults have a varying capacity to elicit antibodies to pneumococcal polysaccharides. A study of 61 ashkenazic family members showed that the capacity to generate anti-pneumococcal polysaccharide IgG was inherited in a mixed, codominant fashion [58]. However, no association between *G2m* allotype [23] and antibody to the polysaccharide was found. Revaccination either with the 23-valent polysaccharide vaccine (single, double dose or 20 times higher dose of a single polysaccharide) or with either Pnc-OMPC or Pnc-CRM failed to elicit antibody production to the relevant polysaccharide in a subject who had initially shown no measurable IgG after vaccination. These individuals also failed to elicit IgG antibody production, and hence the failure to respond was not caused by a failure to switch from IgM to IgG.

The data suggest that a genetically mediated defect in pneumococcal polysaccharide recognition is responsible. However, the genetics of the defect are not understood. It is tempting to speculate that occasional vaccine failures with the 23-valent may be caused at least in part by genetically determined incapacity to make IgG to the capsular polysaccharides.

4. *Neisseria meningitidis*

Meningitis caused by *Neisseria meningitidis* is still a feared disease. Although the total number of cases is small it is a disease which because of its mortality attracts much attention. Here as for Hib and pneumococci a polysaccharide capsule is an important virulence factor, and prospects for prophylactic immunization are good. *N. meningitidis* is an exclusive human pathogen, and transmission is by droplets from colonized upper respiratory mucosal membranes.

There are 12 serogroups based on the structure of the capsular polysaccharide: A, B, C, 29E, H, I, K, L, W135, X, Y, and Z. Approximately 90% of cases of meningitis are caused by strains belonging to serotypes A, B and C. All three serotypes can cause epidemics, but it is usually group A strains that are the causative agent in repeating epidemics in sub-saharan Africa [59]. In Europe and Latin America serogroup B is most prevalent, causing more than 50% of the cases, whereas serogroup C is most prevalent in North America [60,61].

Meningococcal meningitis is spread over all age groups: one-third occurs from 0 to 4 years of age, one-third among 5 to 9 years old, and the final one-third at age 20 and above [62]. Therefore there is a need to vaccinate the whole population. Already in 1913 Flexner published convincing data that serum therapy decreased the fatality rate of meningococcal meningitis [63], and 20 years later Fothergill and Wright observed that there was an inverse relationship between the incidence of meningitis at different ages and the presence of serum antibodies to the causative agent [17].

4.1. Meningococcal glycoconjugates

Purified capsular polysaccharides from serogroups A, C, W135 and Y are marketed products, and as T independent antigens elicit antibody responses with no memory function, with the possible exception of serogroup A [64]. The polysaccharide vaccine from serogroup A elicits an antibody response in infants, and even below the age of 6 months infants appear to be primed [64]. The serogroup C polysaccharide is not immunogenic in children <2 years of age, and development of antibody titers is slow see [61]. Correlates of protection have not been established. However, data suggest that for serogroups A and C serum concentrations of anti capsular antibodies should be >2.0 μ g/ml [61,64]. Glycoconjugates are being developed using the same principles as for Hib and pneumococcal glycoconjugates (see above).

The A and C glycoconjugate vaccines are safe and well tolerated in infants and toddlers and using a 2,4, 6 month schedule for primary immunization all infants

had elicited specific anti-A and anti-C polysaccharide antibody titers $>2.0 \mu\text{g/ml}$ [65–67].

A functional assay is to estimate bactericidal antibodies. A titer of $>1:8$ was seen in all vaccinated, with 47% having equally high titers one year later [65].

In a study in Gambia an interesting and as yet unexplained observation was made: whereas the serogroup C conjugate, as expected, induced memory, no such observation was seen with the serogroup A conjugate [68].

So far all data indicate that meningococcal glycoconjugate vaccines will have as great a chance to be successful as the Hib and pneumococcal conjugates and licensure is expected within the next coming years. Most likely, meningococcal A+C and meningococcal A+C+W135+Y glycoconjugates will be marketed.

4.2. Meningitidis serogroup B

The meningococcal serogroup B polysaccharide, a homopolymer of $\alpha 2$, 8 linked sialic acid residues, is poorly immunogenic in man [69]. Since the same structure is formed in embryonic neural cell adhesion molecules, man is likely tolerant to the polysaccharide structure. It has been suggested that vaccine induced antibodies may interfere with the adhesion molecules causing injury [70], a theory which also has been challenged [71]. The uncertainties have definitely slowed development of a glycoconjugate vaccine, and instead focused interest on outer membrane proteins and protein-complexes. However, antibodies elicited against the serogroup B polysaccharide, although low in titer, are bactericidal [72]. Jennings has modified the group B polysaccharide by replacing the *N*-acetyl groups with *N*-propionyl groups before its conjugation to tetanus toxoid [73]. The resulting polysaccharide mimics a unique protective epitope on the surface of the native group B meningococci and elicits antibodies [74]. Although bactericidal antibody production still only has been shown in mice, the observation holds promise for the development of a human vaccine.

5. Other glycoconjugates

The spectacular success of the Hib conjugates and the likely successes of the pneumococcal and meningococcal glycoconjugates have obviously focused an interest on converting other capsular polysaccharide antigens from T-independent to T-dependent vaccines.

Group B streptococci (GBS) are the leading cause of meningitis and sepsis in neonates in the US. At least six capsular types have been associated with human disease, and type-specific antibodies are responsible for protection via opsonisation of the bacteria by antibodies and complement. Kasper and Jennings have

developed immunogenic glycoconjugates which in animal models elicit protective antibodies [75,76].

Similar attempts are underway to make immunogenic and safe vaccines against the Vi polysaccharide capsule of *Salmonella typhi* [77] and capsular polysaccharide and lipopolysaccharide of *Escherichia coli*, *Shigella sonnei*, and *S. Flexneri* [78,79].

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