CONJUGATE VACCINES – A BREAKTHROUGH IN VACCINE DEVELOPMENT

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Abstract. The encapsulated bacteria *Streptococcus pneumoniae* (the pneumococcus), *Neisseria meningitidis* (the meningococcus) and *Haemophilus influenzae* type b (Hib) are the main causes of purulent meningitis, the peak incidence of which is seen in the first two years of life. The polysaccharide capsule of these bacteria is an essential virulence determinant, and antibodies to it are protective, suggesting that a polysaccharide vaccine could prevent these diseases. The young child is, however, unable to respond with antibody production to these polysaccharides, making such vaccines useless in infancy. Conjugation of the polysaccharide to a protein carrier has proven a way to solve the problem. Immunization of infants with such a Hib conjugate vaccine was shown in 1987 to result in the desired antibody production and protection from Hib meningitis and bacteremia. The Hib vaccine is now a part of national infant immunization programs in large parts of Europe, the Americas and Australia, and has resulted in the virtual disappearance of Hib disease from these areas. A group C meningococcal and 7-valent pneumococcal vaccine, available since 2000, are likewise proving highly effective in preventing bacteremic disease. Further advantages of the conjugate vaccines are their ability to elicit immunologic memory and to reduce asymptomatic carriage of the bacteria, resulting in marked herd immunity. This paper was delivered as a lecture in January 2003 in Bangkok on the occasion of the Prince Mahidol Award for a life's work in the field of vaccinology.

ENCAPSULATED BACTERIA AS CAUSES OF DISEASE

Purulent meningitis is the most severe acute bacterial disease, nearly always lethal if not treated with antimicrobials. The three most common bacteria causing it are *Streptococcus pneumoniae* (the pneumococcus), *Neisseria meningitidis* (the meningococcus) and *Haemophilus influenzae* type b (Hib), in neonatal infection also group B streptococci (GBS) and *Escherichia coli* of capsular type K1. The disease incidence is highest in the first one or two years of life, although the meningococcus is also an important cause of adult disease, often occurring as epidemics, and the pneumococcus in very old or immunosuppressed persons, including the HIV-infected.

In addition to meningitis, these bacteria also cause other forms of invasive (bacteremic) disease and the pneumococcus is also a common cause of other disease entities like otitis media, sinusitis and pneumonia. Recently also Hib has been shown to be an important cause of pneumonia in young children, accounting for an estimated 20% of their pneumonias with X-ray proven consolidation (Mulholland *et al*, 1997). A further common feature is that these bacteria often live on the mucosa of the nose and pharynx as parts of their normal microflora. This colonization or carriage is an important part of the chain of infection, *ie* transmission of the bacteria to new hosts.

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ROLE OF CAPSULE

All these bacteria depend on their polysaccharide capsule for survival in the host; if they loose the capsule they are quickly killed by phagocytes. The same happens if there are antibodies around that bind to the capsular polysaccharide, activate complement and are easily bound by the phagocytic cells. Such antibodies develop as a result of mucosal carriage of the bacteria or, at least in the case of Hib, of other, "cross-reacting" bacteria with polysaccharides resembling the Hib capsule (Robbins *et al*, 1975). Therefore, the antibody concentrations and thereby the protec-

tion from disease that they offer increase with age.

However, this would not fully explain the sharp peak of disease in the first 1-2 years of age. We have only recently learned that this is associated with the inability of the infant and young child to respond to many polysaccharides with antibody production (Mäkelä *et al*, 1995). Polysaccharides are known as T cell-independent antigens, which means that they do not react with T cells but do directly stimulate B cells. This will in most cases lead to antibody production; so why not in the infant? The answer is logical: the relative immaturity of the infant's B cells (Siegrist, 2001). This explains the susceptibility of the young child to the encapsulated bacteria. It also explains why polysaccharide vaccines, prepared from their capsules, are effective in adults and older children but not in infants.

EARLY EXPERIENCE WITH Hib POLYSACCCHARIDE AND CONJUGATE VACCINES

The specific lack of immunogenicity of polysaccharide antigens in infants was clearly demonstrated in an efficacy study with an experimental Hib polysaccharide vaccine conducted in Finland in 1974-75 (Peltola *et al*, 1977). The results showed that children who were older than 1.5 years when receiving the vaccine were protected, while children younger than this were not, and the protection correlated with a clearcut antibody response.

This led to focused new research to find a way to improve the vaccine's immunogenicity in infancy. Several attempts proved fruitless before the advent of conjugation – a technique well known in experimental immunology to make non-antigenic small molecules immunogenic by covalently linking them to a good protein antigen. In 1985 such a conjugate of the Hib polysaccharide with the known vaccine antigen, diphtheria toxoid was shown immunogenic in infants, and two years later its protective efficacy was demonstrated in a randomized field trial (Eskola *et al*, 1985; 1987).

DIFFERENT CONJUGATE VACCINES

Since the first experience with Hib conjugate vaccine, a lot of work has been carried out to learn to understand the properties and function of conjugate vaccines. The Hib polysaccharide has been linked to other proteins (tetanus toxoid TT, a nontoxic mutant diphtheria toxin called CRM 197, meningococcal outer membrane protein complex OMPC) and different conjugation techniques have been used resulting in slightly differing chemical structures at the site of linkage. The general finding has been that all these worked without major differences. On closer look, the conjugate with OMPC has had somewhat different properties, probably because of its content of a small amount of endotoxin. The molecular size of the polysaccharide component could also be varied within a wide frame (Mäkelä *et al*, 1995).

Conjugate vaccines have also been prepared from pneumococcal polysaccharides (7-, 9- and 11-valent vaccines in clinical trials) and meningococci of groups A and C (the group B capsular polysaccharide is not immunogenic probably because of immunologic tolerance due to its similarity to the polysaccharide in the human cell surface protein N-CAM). Overall, their properties are typical of conjugate vaccines as demonstrated by the Hib conjugates, although surely some differences are found in a fine analysis (Mäkelä and Käyhty, 2002).

MAIN IMMUNOLOGIC PROPERTIES OF CONJUGATE VACCINES

The conversion of a T cell-independent antigen to a T cell-dependent one is a critical step. Protein antigens as a rule are T cell-dependent: after uptake and digestion by antigen presenting cells (APC) peptides deriving from them are bound by the MHC class II molecules on the surface of the APC, and this combination is recognized by T cells of the T-helper type. This results in stimulation of the T cell to synthesis of cytokines promoting maturation and proliferation of nearby B cells. If the protein antigen was linked to a polysaccharide (as in the case of conjugate vaccines), it would have drawn B cells recognizing this polysaccharide to the site of vaccine injection, and these cells would now respond to the combined stimulation of the polysaccharide antigen and the cytokines produced by the T cells, with production of antibody to the polysaccharide. In addition, they would proliferate, give rise to a large number of progeny B cells producing more antibody; a fraction of the progeny cells would further develop into memory cells that would respond to a later antigen stimulus with a

rapid production of antibody.

The T cell dependence of the conjugate vaccine is in practice seen as immunogenicity in infants. Also typical of a T cell-dependent antigen is the finding that antibody production is enhanced by subsequent exposure to the antigen. Thus most of the conjugates give a very low or nonexistent response to the first injection, but an enhanced response to the second injection (only the OMPC conjugates result in antibody production after the first dose) (Kurikka *et al*, 1995; Goldblatt *et al*, 1998). The antibody concentrations achieved are clearly higher than obtainable after injection of a polysaccharide vaccine. Further injections even years later result in further enhanced antibody responses due to the memory cells. At the same time the avidity of the antibodies increases, which has been suggested as a means to demonstrate T cell dependence of a new conjugate vaccine candidate (Anttila *et al*, 1999; Usinger and Lucas, 1999).

Quite low concentrations of anti-Hib of the order of 0.15 μg/ml are sufficient for protection from invasive disease (Käyhty *et al*, 1983). It is likely that the high antibody levels are associated with protection from carriage. This means that conjugate vaccines, in contrast to polysaccharide vaccines, are able to prevent or reduce carriage: this effect is quite strong for Hib, and also clearcut with pneumococcal conjugates (Takala *et al*, 1991; Dagan *et al*, 1996). On the other hand, the Hib-OMPC vaccine produces only moderate levels of antibody and has less effect on carriage (Galil *et al*, 1999).

DURATION OF IMMUNITY

From a practical standpoint it is important to know how long the immunity provided by a vaccine will last. With the Hib conjugates we have follow-up data over 8 years; the satisfactory finding was that after an initial decrease of the mean antibody concentrations it soon (after a couple of years) stabilized and did not decrease further (Mäkelä *et al*, 2003). This initially surprising finding appears to be due to frequent new stimuli giving rise, in the presence of memory, to a strong antibody response. Mathematical modeling showed that such stimuli would occur about once in four years. In a vaccinated population in which Hib bacteria had practically disappeared because of the effect of the vaccine on carriage these would be due to cross-reactive bacteria. Because of their frequency, immunity after Hib conjugate vaccine may be lifelong, without the need of further booster doses.

EFFICACY OF Hib CONJUGATES

The protective efficacy of Hib conjugate vaccines has been demonstrated in a number of controlled field trials, both in industrialized [(Finland, UK, USA (Mäkelä *et al*, 1995)] and developing [the Gambia, Chile (Mulholland *et al*, 1940; Levine *et al*, 1999)] countries. In these trials the vaccine has been given together with DTP incorporated in the national infant immunization program, and protection from invasive Hib disease has been seen already after the second dose, reaching 90% or more after the third.

After the first of these trials in Finland in 1985-86, the vaccine was offered to all children coming for their DTP vaccinations, and the coverage reached was over 95%. This resulted in an immediate disappearance of invasive Hib disease from the vaccinated age groups, and after a few years also from older children who had never received the vaccine. This very strong herd immunity effect – protection of also unvaccinated individuals – was clearly due to the effect of the vaccine on carriage of Hib, which in turn reduced the chances of being infected. Later on, modeling of Hib transmission, immunity and invasive disease (Leino *et al*, 2002) and simulation studies in a population model has shown very clearly the importance of carriage on the epidemiology of Hib. On a practical level, Hib has disappeared from areas using conjugate vaccines that are highly effective in preventing carriage but remained at a low level in areas using a vaccine (OMPC-conjugate) less effective towards carriage (Peltola, 2000; Galil *et al*, 1999).

WORLDWIDE USE OF CONJUGATE VACCINES

In the 1990s, Hib vaccine was included in the infant immunization programs in a large part of Europe as well as North America and Australia. In each case, this resulted in a near elimination of invasive Hib disease, and was determined to be highly cost-effective (Lieu *et al*, 2000). The introduction of the vaccine in developing countries has, however, been slow inspite of the good results reported from the Gambia (Mulholland *et al*, 1997). In fact, this latter trial produced new

data that have a major importance to the expected benefits of the vaccine in developing countries. In addition to the expected reduction of invasive Hib infections, the results showed an unexpected 20% reduction of pneumonia defined by consolidation in chest radiograms. The same protection from pneumonia was subsequently shown in a randomized trial in Chile (Levine *et al*, 1999). This means that Hib causes at least 20% of the X-ray proven pneumonias, a much higher figure than thought previously. On this basis one can calculate that Hib vaccine could prevent 20% of the 2 million deaths from acute respiratory infection occurring in children under 5 (Williams *et al*, 2002), *ie* 400,000 deaths annually.

OTHER CONJUGATE VACCINES

As soon as the good results with Hib conjugate vaccines started coming in, it was logical to ask whether the same principle and technology could be applied to other bacteria. The greatest hopes were put in a pneumococcal conjugate vaccine because of the importance of these bacteria in not only invasive disease but also in pneumonia and the very common otitis media. Problems were foreseen in the many serotypes of pneumococci causing these infections. A seven-valent pneumococcal conjugate vaccine given to infants was soon shown to be highly efficacious in preventing invasive infections caused by the vaccine serotypes, and licensed in USA in 2000 (Black *et al*, 2000). It was received enthusiastically, and the present experience, after two years of its use, shows already herd immunity effects in nonvaccinated age groups.

The pneumococcal conjugate is also effective against carriage and otitis media caused by the vaccine serotypes, although less so than against invasive infections (Dagan *et al*, 1996; Eskola *et al*, 2001). The worry here is, however, that other serotypes not represented in the vaccine seem able to take the place of the vaccine serotypes both in carriage and in otitis media. The vaccine serotypes were initially selected to be the ones most common in invasive disease of children and therefore likely to be most virulent; then their replacement (in carriage) by other types would be acceptable, since these would be less virulent and therefore would not cause invasive disease. The early experience supports this view but does not eliminate the possibility of emergence of new, virulent types.

A pneumococcal conjugate vaccine could, in the best case, be a very welcome vaccine for developing countries because of the presumed prominence of pneumococci in pneumonia mortality. Two key questions, however, remain open: first, the efficacy of the vaccine against pneumonia, and second, the proportion of pneumonias and pneumonia deaths due to pneumococci. In the California trial for invasive disease also pneumonia was recorded; the observed reduction was 20% (Black *et al*, 2002). In a randomized trial in South Africa, using a 9-valent study vaccine better covering serotypes in a developing country setting, there was also 20% reduction of X-ray defined pneumonia (Klugman, 2002). In both cases the confidence limits of the estimate are very wide and we do not know how much of the pneumonia was due to pneumococci or, even less certain, due to vaccine serotypes. Thus further data are needed before we can start introduction of pneumococcal conjugate vaccine in developing countries. Then there are other issues to discuss: which serotypes should be included, will there be a separate cocktail for developing versus industrialized countries and how to make the price of the vaccine low enough to be affordable.

Meningococcal conjugate vaccines would be primarily needed to prevent the large epidemics of group A organisms or the smaller, often local, outbreaks of group C meningococci. Both conjugates have been made and shown to be immunogenic and safe (Leach *et al*, 1997). In 2001, the group C conjugate was included in the national immunization program in UK; the effectiveness of the program is already clear (Ramsay *et al*, 2000), and other European countries are considering to follow suit. The group A vaccine has not proven attractive to vaccine manufacturers, clearly because its major markets would be in Africa, but it is now on the agenda of the public-private partnership GAVI, the Global Alliance for Vaccines and Immunization (GAVI, see www.vaccinealliance.org).

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