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Haemophilus influenzae Type b Conjugate and Combination Vaccines

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Summary

Haemophilus influenzae type b (Hib) conjugate vaccines represent a new technology wherein an immunogen is targeted to a specific immune response mechanism. Covalent attachment of the Hib polysaccharide to a protein carrier converts the T cell–independent polysaccharide antigen into a protein-like T cell–dependent antigen. The polysaccharide alone is poorly immunogenic in infants (≤12 months old), and conjugation to a protein carrier results in a protein-like antibody response to the Hib polysaccharide in infants. Conjugate vaccines induce mostly IgG antibodies and immunological memory. Later vaccination or natural exposure then induces a booster response to the Hib polysaccharide.

These conjugate vaccines have dramatically reduced the incidence of Hib disease in many industrialised countries, and also reduce nasopharyngeal carriage of Hib in unvaccinated children in populations in which the vaccine is used. The

Hib conjugates have now been combined with diphtheria and tetanus toxoids and pertussis vaccine to reduce the number of injections required for infants. Finally, the conjugate technology that has permitted the near elimination of Hib disease has now been extended to other invasive encapsulated bacterial pathogens.

Haemophilus influenzae type b (Hib) was the leading cause of invasive bacterial disease in young children under the age of 5 years in most industrialised countries prior to the introduction of conjugate vaccines in 1987.[1] Approximately 1 in 200 children by the age of 5 years developed Hib meningitis, septicaemia or epiglottitis, and 5 to 8% of these died despite effective antimicrobial therapy. Of the survivors, 20 to 30% had some form of lasting sequelae, the most common of which was hearing loss.[2] More than half of all children who develop invasive Hib disease in industrialised countries have meningitis.

The incidence of invasive Hib disease in most developing countries is either unknown or greatly underestimated. Nevertheless, the disease is ubiquitous, occurring in most countries.[3] Some Native American populations in the US and the Aborigines of Australia have had levels of Hib disease 100 times the national average, and such levels may be found in some developing countries.[4,5] Meningitis is the most common form of the disease in developing countries, but peak incidence is often shifted to a lower mean age. Although most forms of Hib disease are seen, epiglottitis, a disease of children over 2 years of age in industrialised countries, is not seen in most developing countries.[3]

1. Role of Antibodies in Protection

1.1 Importance of Capsule

It has been shown by a number of investigators that the Hib capsule is a major virulence factor. Antibodies to the capsular polysaccharide are bactericidal and opsonise the bacteria for phagocytic killing.[6] In a field trial performed in Finland in 1974, the presence of antibodies induced by a *Haemophilus* b polysaccharide vaccine correlated with protection.[7]

1.2 Protective Antibody Levels

The minimum protective level of anti-Hib polysaccharide antibodies at the time of exposure has been estimated to be about 0.15 mg/L. This is based on calculations of the amount of anti-Hib antibody in agammaglobulinaemic children just prior to receipt of an injection of immune globulin.[8] Similar estimates were obtained from passive protection studies in infant rats and from the 1974 Finnish efficacy trial.[7,9] In this trial an anti-Hib polysaccharide antibody level of ≥1.0 mg/L following vaccination with unconjugated polysaccharide vaccine was associated with long term protection against invasive *Haemophilus* b disease.[7] Although the relevance of this antibody threshold to clinical protection after immunisation with a conjugate vaccine is not known, this level continues to be considered as indicative of long term protection.

Passively administered anti-capsular antibodies protect against Hib disease.[10] A bacterial polysaccharide immune globulin (BPIG) has been prepared from the serum of adults immunised with meningococcal, pneumococcal and Hib polysaccharide vaccines. BPIG was given to Native American infants, a population at high risk for Hib disease, at 2, 6 and 10 months of age. The children were protected for at least 4 months after the last administration of BPIG, with a point estimate of efficacy of 86%. One child who received BPIG developed Hib meningitis over 3 months later, and had <0.3 mg/L of anti-Hib polysaccharide antibody at the time of infection.

2. Need for Conjugate Vaccines

The first licensed Hib vaccine, the purified capsular polysaccharide, was approved in 1985.[11,12] The capsule is a phosphodiester-linked ribosyl ribitol polymer, sometimes referred to as PRP. The polysaccharide vaccine was approved in the US based

Table I. Comparison of *Haemophilus* b conjugate vaccines

Vaccine	Manufacturer	Saccharide size	Carrier protein	Spacer (linker)	Formulated to contain (μg/dose)	
					saccharide	protein
PRP-D	Connaught	Polysaccharide	Diphtheria toxoid	Adipic hydrazide (6-carbon spacer)	25	18
PRP-CRM	Lederle-Praxis	Oligosaccharide	Diphtheria protein (CRM)	None (amide)	10	25
PRP-OMPC	Merck	Small polysaccharide	Meningococcal protein	Thioether (bigeneric)	15	200
PRP-T	Pasteur Merieux	Polysaccharide	Tetanus toxoid	Adipic hydrazide (6-carbon spacer)	10	24

upon a randomised efficacy trial in 100 000 children conducted in Finland between 1973 and 1977, with an estimated efficacy of 90% (95% confidence limits 55 to 98%) for children ≥18 months of age.[7] The vaccine, however, proved less effective in the US than in Finland, and was not used to any extent outside the US and Canada.[13] Following approval, a series of post-licensure case-control studies in the US gave quite variable results, ranging from no protection to 88% effectiveness (see section 6.1).

The uncertain effectiveness of the Hib polysaccharide vaccine and reports of vaccine failures provided the impetus to approve in 1987 the first Hib conjugate vaccine, PRP-D, produced by Connaught Laboratories for use in children ≥18 months old.[11,14] Interestingly, many of the Hib polysaccharide vaccine failures were due to an apparent selective defect in the immune response to the native polysaccharide.[15] However, when these children were reimmunised with a Hib conjugate vaccine they responded normally.[15]

3. Chemistry of Licensed Conjugate Vaccines

Hib conjugate vaccines represented a new application by Robbins and his colleagues in 1980 of a biotechnological process of chemically attaching saccharides to protein carriers which was developed 50 years earlier.[16,17] There are now 4 different Hib conjugate vaccines licensed in the US,[11,18] all different, having their own physical, chemical and immunological characteristics (table I). A detailed review of the conjugation chemistry and quality control used in these vaccines was recently published.[19]

The first conjugate, PRP-D, consisted of size-reduced Hib polysaccharide attached through a 6-carbon spacer, adipic hydrazide, to diphtheria toxoid using the procedure of Schneerson et al.[17] Later the conjugation chemistry was modified and the adipic acid dihydrazide spacer was added first to the polysaccharide, which was then conjugated to the purified protein.[20] Attachment of the spacer first to the polysaccharide was more generally applicable, because initial attachment to the protein reduced protein solubility and conjugation efficiency. The vaccine PRP-T utilised the improved chemistry to covalently link Hib polysaccharide to tetanus toxoid.

The PRP-CRM vaccine does not contain Hib polysaccharide, but rather oligosaccharides of about 20 repeat units derived by periodate oxidation, attached directly to a nontoxic mutant form of diphtheria toxin known as CRM_{197} as pioneered by Anderson et al.[19,21] The ratio of oligosaccharide to protein was found to be critical for optimal antibody response.[11,21]

Compared with the other conjugate vaccines, PRP-OMPC has a number of unique properties. The protein carrier is not a component of the diphtheria/tetanus/pertussis (DTP) vaccine, but consists of lipopolysaccharide-depleted meningococcal outer membrane vesicles attached to size-reduced Hib polysaccharide through a thioether linkage.[19] In this process, separate linkers are attached to both the protein and the Hib polysaccharide, followed by fusion of the linkers.

Although there are a number of other Hib conjugate vaccines produced in other countries, most resemble one of the above vaccines.

4. Immunology and Kinetics of Immune Response

4.1 Immunology

The nonconjugated Hib polysaccharide, like most polysaccharides, is capable of stimulating B lymphocytes to produce antibody without help from T lymphocytes (T cell–independent response).[22] Responses to protein antigens are augmented by helper T lymphocytes (T cell–dependent response). Covalent attachment of the Hib polysaccharide to a protein carrier converts the T cell–independent polysaccharide into a T cell–dependent antigen. The ability of a conjugate to recruit helper T lymphocytes into the immune response helps explain why an infant responds to the *Haemophilus* b conjugate vaccine, but not to the polysaccharide on primary immunisation.[23]

The T cell–dependent nature of the immune response also results in induction of immunological memory, leading to an anamnestic or booster response on re-exposure to the native polysaccharide.[24,25] Granoff et al.[24] found that immunisation during infancy primed for a memory response when infants were revaccinated with the polysaccharide at 12 months of age. In contrast, infants who have not been immunised during infancy and receive the polysaccharide vaccine at 12 months have a negligible antibody response. Barbour et al.[25] found evidence that the primary immunisation series during infancy primed children to respond with a booster response upon later Hib colonisation. Other features of a T cell–dependent response such as predominance of IgG1 and maturation affinity are also seen with conjugate vaccines.[22]

Bactericidal titres in post-vaccination sera against Hib are strongly correlated with the affinity or functional avidity of the sera.[26,27] Those children with low-affinity antibody after immunisation showed little increase in bactericidal activity. Although there was a direct and significant correlation between bactericidal activity and functional avidity, a wide range of anti-Hib polysaccharide antibody concentrations were found to have equivalent bactericidal titres, indicating that multiple factors contribute to the observed bactericidal titre.[26] The *in vivo* biological importance of antibody avidity is not clear.

4.2 Kinetics

As might be expected from the large differences in composition among the 4 conjugate vaccines (table I), the kinetics of the antibody responses of infants to these vaccines are quite different (fig. 1). Two different methods have been used to compare Hib conjugate vaccines:

- geometric mean titre using a standardised radioimmunoassay
- rate of seroconversion to 1 mg of anti-Hib antibody/L.

The latter is more important since it is an indication of the percentage of vaccine recipients expected to have long term protection.

A number of comparative trials of the different Hib conjugate vaccines in infants have been conducted in the US and Finland.[28–32] Although PRP-D gave excellent protection in children ≥18 months of age,[33] in each of the comparative trials it was clearly less immunogenic in infants than the other Hib conjugates. Upon completion of the primary immunisation series at 2, 4 and 6 months of age (US schedule), PRP-CRM and PRP-T consistently induce the highest immune responses and seroconversion rates in a variety of populations. However, one problem with these vaccines is that PRP-CRM induces relatively little antibody until after the third injection, and PRP-T requires at least 2 doses to stimulate a reasonable response. Therefore, they both act as typical T cell–dependent immunogens, requiring carrier priming for an optimal antibody response.[33] They induce mostly IgG1 of high avidity.[26]

Unlike the other Hib conjugate vaccines, a single dose of PRP-OMPC elicits in 2-month-old infants a strong clinically important IgG and IgM response. However, only modest increases in re-

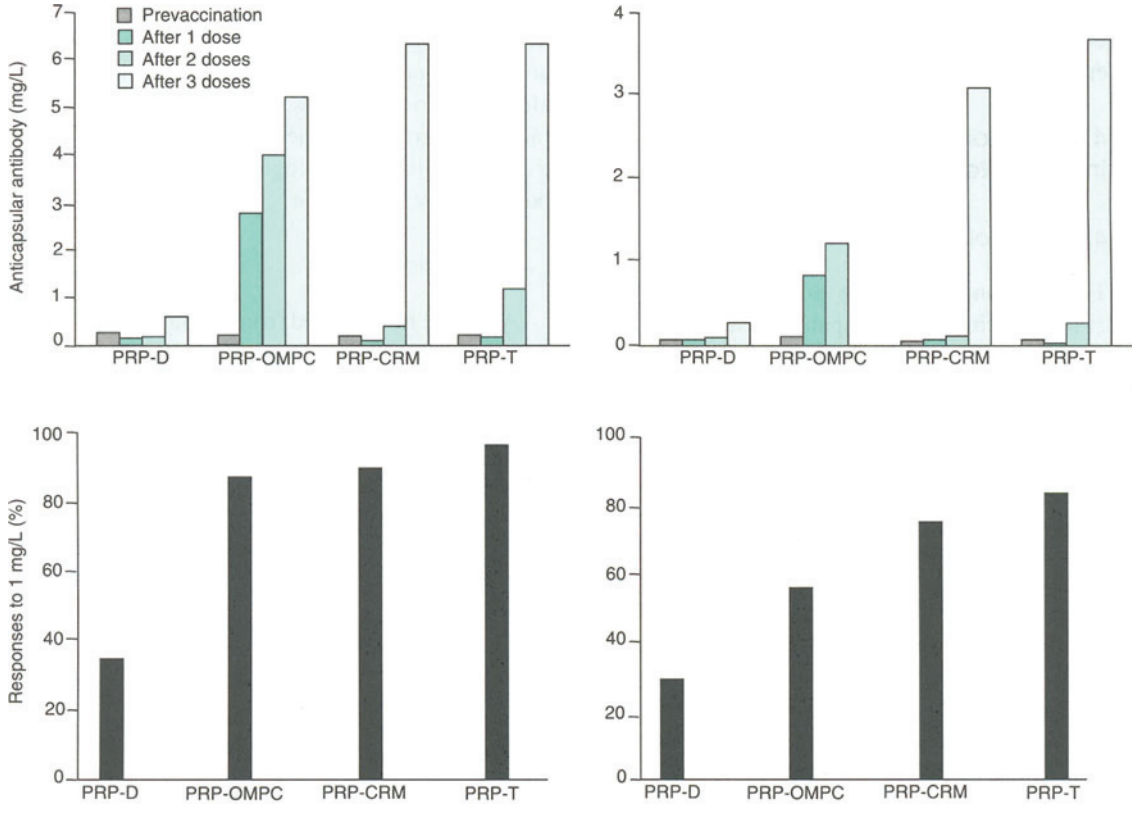

Fig. 1. Antibody responses of infants to different Hib conjugate vaccines. The left-hand column shows data from a study in Minnesota, Missouri and Texas[18,29,30]; the right column shows data from a study in Tennessee.[28] The top row shows the levels of anticapsular antibody before vaccination and after 1, 2 and 3 doses of vaccine. The bottom row shows the percentage of vaccine recipients with anticapsular antibody levels ≥1 mg/L after vaccination.

sponse are observed after the second dose (in the US, PRP-OMPC is administered at 2 and 4 months of age). Induction of significant antibody levels after a single injection may be of importance where there is a high incidence of Hib disease in early infancy, as in some Native American populations. Use of meningococcal outer membrane vesicles as the protein carrier may be responsible for the rapid immune response (fig. 1).[34] The OMPC carrier consists of lipopolysaccharide-depleted outer membrane vesicles from a strain of group B *Neisseria meningitidis*. The major porin protein in these vesicles was shown by Liu et al.[34] to be a human peripheral blood lymphocyte mitogen. The toxoid carriers in the other conjugates are not mitogenic. Thus, the PRP-OMPC vaccine appears to have both T cell–independent and T cell–dependent properties.[14,34] Whereas conjugate vaccines utilising a toxoid carrier protein appear to require carrier priming, PRP-OMPC does not.[35]

4.3 Mix and Match

With no information available on the interchangeability of Hib conjugate vaccines, the conservative recommendation was that the same vaccine be used for the primary immunisation series. However, due to population mobility, a child may not return to the same medical care provider to complete his/her pri-

mary immunisation series. Studies now show that any Hib conjugate vaccine recommended for infant administration can be used.[18,36] Any combination of 3 doses of Hib conjugate vaccine will stimulate an adequate immune response. A sequential combination of PRP-OMPC for the first dose and then either PRP-CRM or PRP-T for the second and third doses resulted in protective antibody levels after the first dose and very high peak antibody levels at 7 months of age.[37]

4.4 Booster Dose

In many countries, although not all, a booster dose with any of the Hib conjugate vaccines shown in table I is recommended between 12 and 18 months of age. Studies have shown that any Hib conjugate can be used for the booster.[38] A booster is recommended because there is a decay in peak antibody levels, with an estimated half-life of between 12 and 16 weeks,[31] and it has been desirable to provide protection up to 6 years of age. The immunisation schedule in the UK is at 2, 3 and 4 months of age with no booster, but it is not yet clear whether this strategy provides for solid protection up to 6 years of age, based on the epidemiology of Hib disease prior to Hib vaccine.

Interestingly, PRP-D used as a booster following use of any of the other vaccines in infants produced post-booster antibody levels that equalled or exceeded those obtained with the vaccine used for the infant immunisation.[38] Individual children who have essentially no measurable antibody response (<0.1 mg/L) after the full 2- or 3-dose infant immunisation series are primed to respond as well as those who responded strongly as infants.

5. Efficacy Studies Supporting Approval

Two Hib conjugate vaccines, PRP-CRM and PRP-OMPC, were approved in the US in 1990 for routine administration to infants on the basis of controlled efficacy trials (table II).

5.1 PRP-CRM

The PRP-CRM trial was conducted in a large health maintenance organisation in Northern California.[39] Over 20 000 infants received PRP-CRM at 2, 4 and 6 months of age. The trial was not placebo-controlled; rather, there were 2 control groups totalling 30 500 infants comprising:

- those born during the first 6 days of each month, who were not offered the vaccine
- those who were offered the vaccine but refused.

After 1 year of follow-up, efficacy was 100%. There was also clear evidence of protection after the second dose, but not after 1 dose.

5.2 PRP-OMPC

The PRP-OMPC efficacy study was a double-blind placebo-controlled trial conducted in a Native American population.[40] This permitted a much smaller study population, but there was concern about conducting a trial in such a high-risk population, since PRP-D had failed to protect in a

Table II. Efficacy of *Haemophilus* b conjugate vaccines in infants

Vaccine	Study population	Study group	Number of participants	Number of cases	Efficacy (%) [confidence interval]	Reference
PRP-CRM	California, USA	Unvaccinated	18 862	12	100 (68-100)	39
		Fully vaccinated[a]	20 800	0		
PRP-OMPC	American Native	Placebo	1 929	14	93 (53-98)	40
		Fully vaccinated[b]	1 913	1		
PRP-T	Oxford, UK	Unvaccinated controls	16 484[d]	18	95 (75-100)	42
		Fully vaccinated[c]	15 499[d]	1		

a Immunisation at 2, 4 and 6 months of age.

b Immunisation at 2 and 4 months of age.

c Immunisation at 2, 3 and 4 months of age.

d Child-years at risk.

similar population.[41] In children immunised with PRP-OMPC at 2 and 4 months of age and followed to 18 months of age, only 1 case occurred for a point estimate of efficacy of 93%. No cases occurred in vaccine recipients between the first and second doses, whereas several cases occurred in the placebo group.

5.3 PRP-T

PRP-T was approved in the US in 1993 based upon immune correlates of protection,[11] but was approved in the UK in 1992 on the basis of a controlled community intervention trial in the Oxford region (table II).[42] The vaccine was offered in 4 districts but not in 4 adjacent districts, and was administered using an accelerated schedule at 2, 3 and 4 months of age. No booster immunisation was given. The point estimate of efficacy involving over 25 000 fully vaccinated children was 95% after 1 to 2 years of observation. The protection observed through the second year of life without a booster and the use of the accelerated schedule provided information for the World Health Organization to consider for incorporation of Hib conjugate vaccines in their Expanded Program of Immunisation.

5.4 Immunological Surrogates of Efficacy

Once a vaccine is approved, it becomes ethically difficult to design an acceptable placebo-controlled clinical trial to demonstrate efficacy of another Hib conjugate vaccine. Immunological surrogates should therefore be used to demonstrate equivalence between a new vaccine and the existing licensed Hib conjugate vaccines.

Some immunological surrogates of an effective Hib conjugate vaccine are presented in table III. Studies of the 4 different Hib conjugate vaccines have shown a number of common features that clearly differentiate the immune responses to conjugate vaccines from those to the unconjugated Hib polysaccharide. These include:

- induction of antibodies in infants at an age when they do not respond to the free polysaccharide
- induction of higher levels of IgG1 relative to IgG2

Table III. Some immunological surrogates of an effective *Haemophilus* b conjugate vaccine to be considered in evaluation of new vaccines

1. Randomised comparative immunogenicity studies with currently licensed vaccines in infants
2. Measurement of antibody persistence after the primary immunisation series up to age of the recommended booster dose
3. Determination of whether the conjugate vaccine primes infants for a subsequent booster response to the native polysaccharide given 6 months or more after primary immunisation
4. Comparison of IgG, IgM and IgG subclasses following the primary immunisation series to those reported for licensed vaccines
5. Demonstration of functional capacity of conjugate-induced antibodies in infants by measurement of opsonic or bactericidal activity

- priming of infants for a booster response to the native polysaccharide.

A conjugate vaccine acting as a T cell–dependent antigen will induce immunological memory, whereas the native polysaccharide will not. Since long term protection following immunisation with the conjugate may depend upon a booster response to the native polysaccharide, induction of immunological memory by a new conjugate should be experimentally confirmed by administration of the polysaccharide at least 6 months after completion of the primary immunisation series. As shown in figure 1, conjugate vaccines can differ from each other in the magnitude and duration of the initial response induced.

A new vaccine should be shown to induce functionally important antibodies. Both opsonic and bactericidal antibodies are important, and have been shown to correlate with one another.[43] It is likely that opsonic activity alone is sufficient, because individuals with deficiencies in the late complement components do not appear at increased risk of Hib disease, as they are with meningococcal disease.

6. Effectiveness in Clinical Use

6.1 Decline in Disease in the US

Since introduction of Hib conjugate vaccines for use in infants, dramatic declines in Hib disease

have been documented in a number of industrialised countries.[44-50] The effect of the polysaccharide vaccine and the later conjugate vaccines on the incidence of Hib disease was examined by intensive surveillance of children <5 years old in Dallas, Texas and in Minnesota from 1983 to 1991.[45]

In the period between 1985 and 1987, when only the Hib polysaccharide vaccine was available for use in children 2 to 6 years old, no decrease in Hib disease occurred in Minnesota and a 35% decline was noted in Dallas. From 1987 to 1990, when the Hib conjugates were approved only for children ≥18 months old, the incidence of Hib disease among children 18 to 59 months old decreased by 91 and 86% in Minnesota and Dallas, respectively. At the same time, the incidence in children <18 months old also decreased by 33 and 45% in Minnesota and Dallas, respectively, suggesting herd immunity.

The Northern California Kaiser Permanente health maintenance organisation achieved high levels of Hib vaccine (PRP-CRM) coverage in their infant population starting in 1991.[50] As a consequence, Hib disease was essentially eliminated in this population. However, cases continued to occur in children who received less than the recommended 3 doses of PRP-CRM. Some cases of Hib disease are reported to the US Food and Drug Administration (FDA) through the Vaccine Adverse Event Reporting System (VAERS) as vaccine failures.[51] On investigation, we found that most cases were in young children who had not completed their primary immunisation series of 2 or 3 doses of Hib vaccine.

In a retrospective study of the impact of Hib vaccines on the incidence of Hib meningitis at the Arkansas Children's Hospital in Little Rock, a graduated decline correlated with the stepwise decrease in the age of Hib immunisation.[48] From 1985 to 1987, 27.3 (standard deviation ± 4) cases of Hib meningitis were treated at the hospital each year, compared with 19.0 ± 2 per year from 1988 to 1990, and 1.7 ± 2.9 per year from 1991 to August 1992.

Studies by Michaels and Ali[49] showed that the incidence of Hib meningitis in Pittsburgh, Pennsylvania, was decreasing several years before routine Hib vaccination began. However, this trend was not seen in California nor by investigators from the Centers for Disease Control (CDC) in Atlanta, Georgia, in their survey of 20 states in the National Bacterial Meningitis Reporting system for 1980 to 1991.[46,50] The CDC study did report a direct correlation between the decrease in the incidence of Hib disease and increased conjugate vaccine distribution. They also observed a marked decline in Hib disease in children <1 year old prior to approval of the vaccine for use in that age group. This is most likely to be related to reduced acquisition rates and transmission of Hib in populations in which Hib conjugate vaccines are used.[47]

6.2 Decline in Disease in Other Countries

In the Netherlands PRP-T is administered along with other paediatric vaccines at 3, 4 and 5 months of age with a booster at 11 months.[52] Their programme started with children born in April 1993. The incidence of Hib meningitis was compared for 1 year before and after introduction of the vaccine. There were 14 cases (22/100 000) in the year before introduction, compared with no cases in children who had received at least 2 injections of PRP-T. A similar decline (95%) was seen in England, where children receive either PRP-T or PRP-CRM at 2, 3 and 4 months of age with no booster.[42]

In Germany, a less marked decline was observed than in The Netherlands.[53] In Germany, children receive 2 doses during the first 6 months and a booster at 15 months of age with any of the 4 vaccines shown in table I. The incidence in children <5 years old declined from 23/100 000 to approximately 2/100 000 per year. Unlike other countries, PRP-D is commonly used in Germany and most of the cases of vaccine failure were in children who had received PRP-D. This suggests a lower overall effectiveness of PRP-D.

A marked reduction in Hib disease was found in Lucerne, Switzerland, following introduction of PRP-D for infants at about the same time as in Germany.[54] A further reduction was noted after more immunogenic vaccines were introduced, providing

additional evidence that vaccines able to induce higher initial antibody levels provide better long term protection.

McIntyre et al.[55] found a direct correlation with the increase in use of Hib conjugate vaccine in the Sydney region of Australia and the decline in all forms of Hib disease. Prior to introduction of the vaccine, the incidence was approximately 50/100 000 per year in non-Aboriginal children <5 years old, and 460/100 000 in Aboriginal children.[56] The PRP-OMPC vaccine, the first of 3 now available, became available for infants at 2 months of age in January 1993. By mid-1994, significant declines ($p < 0.001$) were seen in children 6 to 59 months of age compared with levels in 1991 and 1992.

Some data exist on effectiveness of Hib vaccination for specific diseases. Takala et al.[57] examined the impact of Hib conjugate vaccines on the incidence of epiglottitis in Finland. Since 1990, all Finnish children have been offered PRP-T at 4, 6 and 14 to 18 months of age. Prior to initiation of vaccination, the incidence of epiglottitis was approximately 13/100 000 per year in children <5 years old, and by 1992 it had decreased very significantly to 0.3/100 000.

Thus, in all countries where Hib conjugate vaccines have been introduced and administered to a major portion of their infant population, and where prevalence studies have been done before and after introduction of the vaccine, marked declines in Hib disease have been documented.

6.3 Effect on Carriage

Unlike the Hib polysaccharide vaccine, conjugate vaccines reduce rates of disease in unimmunised children, and studies in a number of countries show a direct correlation between vaccination and decreased carriage.[44,47,58] Between 1987 and 1989, Murphy et al.[45] conducted a prospective study among children attending day care centres in Dallas, Texas, to evaluate the effect of vaccination on prevalence of Hib carriage. They found that unvaccinated, Hib polysaccharide–vaccinated and PRP-D–vaccinated children had asymptomatic carriage rates of 18, 21 and 7% respectively. Thus, while the polysaccharide vaccine had no effect upon carriage, PRP-D reduced carriage by 64%.

Barbour et al.[47] studied Hib colonisation in 2 neighbouring counties in England, in one of which infants had received PRP-T. At 12 months of age, vaccinated children were significantly (0.5 versus 5.6%) less likely to be carriers, a 90% reduction. Vaccination also resulted in lower carriage rates in siblings of vaccinated children, correlating with the observation that the siblings also had lower rates of Hib disease. Approximately 4 years later, carriage rates in vaccine recipients and controls were not significantly different (6.7 versus 5.0%). At that point, 49 and 20% of vaccine recipients and controls, respectively, had anti-Hib polysaccharide antibody levels of ≥1 mg/L.[25]

7. Combination Vaccines

Hib conjugate vaccines are now recommended in many industrialised countries for routine immunisation of infants, adding yet further injections to the child's schedule of immunisation. It is thus advantageous to combine Hib conjugate vaccines with other vaccines, such as DTP, that are routinely administered to infants. The smaller number of injections would potentially improve both parental and physician acceptance.

There are now 2 different combination vaccines available in many industrialised countries: Tetramune® (Lederle Praxis Biologicals) and freeze-dried PRP-T (Pasteur Merieux). Tetramune® is a combination of DTP (whole cell pertussis) and PRP-CRM. Freeze-dried PRP-T may be reconstituted either with DTP or with DTP containing inactivated polio vaccine (IPV). In each case the safety and immunogenicity of the combined vaccines has been shown to be equivalent to those of the DTP and Hib components administered separately.[18] Studies in Beer-Sheva, Israel, by Dagen et al.[59] demonstrated that administration of PRP-T reconstituted with DTP plus IPV was both safe and immunogenic. Compared with DTP plus IPV alone, the antibody responses to each of the vaccine components were not different, except for minor de-

creases in tetanus antitoxin levels and pertussis agglutinin titres.

Additional combination vaccines formulated around DT plus acellular pertussis vaccines, as well as vaccines containing hepatitis B antigens, are under investigation.[18]

8. Conclusions

The striking success and safety record of Hib conjugate vaccines in preventing the various forms of Hib disease represents an application of immunology discovered over 60 years earlier. Conversion of the Hib polysaccharide from a T cell–independent antigen to a T cell–dependent antigen allows an otherwise very poor immunogen to induce high levels of protective antibodies in infants. This technology, proven with Hib, has now been applied to the prevention of other invasive bacterial diseases of young children caused by encapsulated pathogens, including *Neisseria meningitidis* and the pneumococcus.

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