Conjugate Vaccines and the Carriage of *Haemophilus influenzae* Type b

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Pharyngeal carriage of *Haemophilus influenzae* type b (Hib) is important in the transmission of Hib organisms, the pathogenesis of Hib disease, and the development of immunity to the bacterium. The remarkable success of current vaccination programs against Hib has been due in part to the effect of conjugate Hib vaccines in decreasing carriage of Hib. This review explores evidence for this effect, and discusses the possible mechanisms of the mucosal influence of Hib conjugate vaccines.

Many junior doctors today have not had occasion to treat a child for *Haemophilus influenzae* type b (Hib) disease. The remarkable absence of cases of this disease is due to the use of conjugate vaccines. In countries with established Hib vaccination programs the incidence of disease has declined sharply (1). In fact, in some countries protection provided by Hib conjugate vaccines appears to extend to unvaccinated infants in the population (2,3). This phenomenon has been attributed to the conjugate vaccines' effect of decreasing Hib colonization; however, few controlled studies have been conducted in this area and many aspects of the Hib conjugate vaccines' influence on carriage remain speculative. This review explores Hib carriage in the context of current efforts at elucidating the effect of conjugate vaccines on Hib within human mucosae.

What Is Carriage?

A carrier is a person who harbors a specific infectious agent in the absence of clinical illness with or without a detectable immune response (4). The carrier state may reflect carriage of the organism in the incubation period before clinical symptoms appear, during an illness, or after recovery from illness. The carrier state may be short or long, and it may be intermittent or continuous. Carriers may spread infection to others. Latency should be distinguished from the carrier state, in that a latent organism is not transmissible. Hib carriage can be synonymous with Hib colonization and is defined as the presence of viable Hib organisms in the human pharyngeal mucosa. This definition depends on the sensitivity and specificity of the process used to identify viable bacteria in a healthy host.

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The Detection of Carriage

Because Hib carrier status is usually determined by culture techniques, the efficiency of every step in the microbiologic investigation must be maximized. Potential problems in the microbiologic detection of Hib include consistency of swabbing technique, which is difficult to maintain; survival of the organism during transport on the swab to the culture medium; and the morphologic similarity of *Haemophilus* species on solid media, their fastidious growth requirements, and the abundance of other bacteria in the specimens.

The development of an antiserum agar culture method by Michaels et al. (5) has overcome some of the problems in isolating Hib from a sample containing mixed flora. This method has been successfully modified for use in large scale studies (6). Pharyngeal swabs are plated onto a transparent solid medium impregnated with high-titer antiserum for the serotype b capsular polysaccharide. Isolated Hib colonies in a mixed culture are readily recognized on this medium because they are surrounded by halos of antigen-antibody precipitate.

Most surveys of Hib carriage use Michaels' antiserum agar method to establish carrier status. Before this method became available, the diversity of culture methods used to isolate Hib from pharyngeal swabs was a major factor complicating the interpretation of data from different studies. In most investigations, difficulties in isolating Hib may have contributed to an underestimate of Hib carriage rates.

The Epidemiology of Hib Carriage

The most important factors contributing to the epidemiology of Hib carriage are social and demographic. The probability of Hib carriage in a young

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child seems closely related to the likelihood of exposure to the organism.

Most surveys agree that nasopharyngeal or throat cultures recover Hib in 3% to 5% of young children (7,8), with age being a prominent determinant of Hib carriage rates (9). Carriage rates are low in the first 6 months of life, reach a maximum between the ages of 3 and 5 years, and gradually decline in adulthood. Under circumstances of crowding or Hib disease within a closed population, the carriage rate may be substantially higher (7,10,11). Hib carriage rates among children increase with the number of siblings in a family (9), and in the United States, especially after the occurrence of Hib disease in a child care center, carriage rates of 50% have been found (12). The influence of concurrent upper respiratory symptoms on Hib carriage rates remains controversial, and studies differ in their findings on the influence of season, sex, and race on carriage rates. Most studies find no association of Hib carriage with these factors. Antimicrobial therapy affects Hib carriage; in particular, if given appropriately, rifampicin may eliminate throat carriage of Hib and reduce the risk for secondary disease among contacts (13).

Longitudinal studies of children further characterize Hib carriage. Although many children may be transient carriers of Hib (14), carriage tends to persist for many weeks or months (10,12,13). It seems that close contact and generous exchange of respiratory secretions is required for the transmission of Hib between hosts. Even when the contact between a known carrier and a susceptible child is intimate, spread of Hib occurs slowly over weeks or months (7,8). Bijlmer has suggested that village population dynamics and living conditions in the Gambia contribute to different kinetics of colonization and transmission of Hib, as persistence of carriage in Gambian children is short-lived (15).

Hib Carriage and the Transmission of Disease

Since most patients with Hib disease have not had contact with a person who had invasive disease, and the organism has no known reservoir outside humans, asymptomatic carriers have been recognized as the major source of infection. The relationship between carriage rates and the risk for disease is not simple. The spread of infection in the presence of low carriage rates has been described (16), and no overt disease has been reported despite high carriage rates (7,17). Furthermore, organisms isolated from the pharynx appear to lack certain virulence attributes found in organisms isolated from patients with invasive disease (18).

Like surveys of Hib carriage, epidemiologic surveys of Hib disease have found that rapid dissemination of Hib strains has not been the rule, although the temporal clustering of several episodes of systemic disease in child care centers has occurred. The secondary attack rate for household contacts is approximately 500 times higher than the endemic-disease risk for the general population (1). The high frequency of Hib carriage in homes and child care centers of patients who have Hib disease suggests either that a high concentration of carriers precedes and increases the probability of cases, or that affected children are potent sources of infection for others in close contact.

Although encapsulation enhances the ability to survive the dehydrating stress that occurs during transfer between hosts (20), a great deal remains unknown about the transmission of Hib. What are the modes of transmission between hosts? How long can organisms remain viable between hosts? Is there a threshold colonizing population or dose of organisms to ensure successful transmission? Is there any correlation between the size of the infective dose or the number of carried organisms and the likelihood of invasive disease?

Hib Carriage and the Pathogenesis of Disease

The accepted pathogenesis of invasive Hib disease begins with the pharynx as the portal of entry. The infant rat model of *H. influenzae* meningitis has been used to study the early pathogenic events of Hib disease. Infant rats contracted meningitis after intranasal challenge with Hib (21). There was an age-dependent susceptibility to meningeal invasion (22), and bacteremia followed the nasopharyngeal inoculation of organisms. The incidence of bacterial meningitis, irrespective of rat host age, was directly related to the intensity of bacteremia (22). To reach the bloodstream, this nonmotile bacterium must pass through or between epithelial cells, penetrate the basement membrane and subepithelial tissue, and enter the endothelium of a blood vessel. The manner by which this occurs remains under investigation.

Untreated, Hib meningitis and epiglottitis are fatal in most cases. The death of the host is disadvantageous to the infecting bacterium since death terminates transmission and propagation of organisms. Vascular invasion by Hib may be circumstantial or accidental rather than the result of evolutionary advantage.

Hib Carriage and the Development of Immunity

The capsular polysaccharide of Hib is a linear polymer of ribose, ribitol, and phosphate (23) and is called polyribosylribitol phosphate (PRP). PRP is the principal determinant of virulence of Hib (24,25) and a target for antibody-mediated immunity. Considerable evidence indicates that antibody to PRP is a principal protective host factor (26).

At birth, maternal anti-PRP IgG confers protection; however, as the level of maternal antibody in the infant declines, the risk for and incidence of Hib disease rise. As children approach 2 years of age, their own antibody to the capsular polysaccharide begins to appear. The antigenic stimulus for this age-dependent development of bactericidal activity may be through mucosal exposure to Hib or to other cross-reacting antigens (27).

High serum anti-capsular antibody concentrations are associated with the Hib carrier state in children older than 18 months (17,28), but how colonization increases serum anti-PRP antibody concentrations remains unclear. Hib antigen may be absorbed across the mucosa, or the whole organism may traverse this barrier during colonization, causing low grade asymptomatic bacteremia and a systemic antibody response. It is possible that Hib organisms are phagocytosed by lymphoid cells, in the pharyngeal mucosa, that may act as antigen-presenting cells (29), leading to the production of specific serum or mucosal antibodies, with or without direct vascular invasion by the organism. Other mucosal surfaces, such as the gastrointestinal mucosa, may play a role in anti-PRP immunogenesis. The incidence of bacteremia and meningitis in infant rats was significantly lower for rats fed with *Escherichia coli* that possessed K100 capsular antigen (cross-reactive with type b capsular antigen) than for rats fed with *E. coli* K92 or saline (30).

Many have argued that the high prevalence of anti-PRP antibodies and the low rates of Hib carriage in children point to sources other than Hib that give rise to serum anti-PRP antibodies. However, the technical difficulties in identifying Hib carriage may have led to an underestimate of carriage rates. With more sensitive culture techniques, it now seems more plausible that Hib carriage or infection could account for the acquisition of natural immunity.

Vaccination and Hib Disease

The initial development of vaccines against Hib in the late 1960s was prompted by three major concerns: the high case-fatality rate of Hib disease, the high incidence of central nervous system sequelae in children surviving Hib meningitis, and the gradual emergence of strains resistant to preferred antibiotics. Because serum anti-PRP antibodies were known to be a principal protective factor in the host, efforts were made to increase the antibody concentration by active immunization with a vaccine composed of purified capsular polysaccharide. While the PRP vaccine was effective in protecting healthy children 18 months of age and older against invasive Hib disease (with an estimated efficacy of 90%), it did not protect younger children who have the highest incidence of Hib disease (31).

The explanation for the vaccine's inability to protect younger children lies in the chemistry of the capsular material. PRP, a heteropolymer of pentose sugars, does not elicit a T-cell–dependent immune response and is not an efficient immunogen, especially in young children. Carbohydrate antigens, such as these capsular polysaccharides of encapsulated bacteria, are characterized as T-cell-independent type 2 (TI-2) antigens. However, for many antigens the classification T-cell–independent or T-cell–dependent refers more to the pattern of antibody response than to the involvement of T-cells in eliciting that response. In the very young, the antibody response to T-cell–independent antigens is low, consists of a high proportion of IgM antibody, and there is no booster response to repeated doses of antigen.

Converting polysaccharide into a T-cell–dependent antigen, to which infants can respond, requires the covalent linkage to protein molecules, thus producing a conjugate vaccine. Studies of the different conjugate vaccines have confirmed that conjugation results in a T-cell–dependent response (32). Even though the protein carriers and covalent linkages are biochemically and structurally different, they appear to be more immunogenic than pure PRP vaccines. Conjugate vaccines can confer protection against Hib in infants under 6 months of age (33).

Four conjugate vaccines have undergone clinical evaluation: PRP-D (Connaught Laboratories), PRP-T (Pasteur-Merieux), PRP-OMPC (Merck Sharp and Dohme), and HbOC (Praxis Biologicals). Each of these vaccines is distinguished by its carrier molecule, the size of the hapten saccharide, the type of linkage between hapten and carrier, and the ratio of polysaccharide to protein. These physicochemical differences between the vaccines influence their immunogenicity. Additionally, dose variation, such as the age at which the primary series of two or three immunizations is given and the interval between each dose of vaccine, may affect the level of anti-

body response and/or the protection provided.

The concentration of vaccine-induced serum antibody against Hib needed for protection is not precisely known. The issue is complicated as similar concentrations of antibodies may vary in functional activity (34). The maintenance of a threshold concentration of serum anti-PRP antibody may not be necessary if immunized children are primed for an effective booster response on exposure to Hib.

Efficacy studies have shown that PRP-D, HbOC, PRP-OMP, and PRP-T can prevent more than 90% of Hib disease (1,33,35,37). However, PRP-D proved to be ineffective in preventing Hib disease in an efficacy trial among Alaskan children (38). Thus, the choice between the conjugate vaccines may be critical only in populations in which infection pressure is very high, and the age of greatest incidence of disease is low. In these populations, conjugate vaccines with high immunogenicity at the first dose may be the best to use (39), so that the youngest children at the highest risk are afforded some protection at the earliest opportunity. In most populations, the choice of vaccine has to be based on other factors, the most important of which is cost.

Conjugate Vaccination and Hib Carriage

After a 4-year national immunization program for infants in Finland, Takala et al. (40) reported that Hib colonization was less prevalent among 327 children vaccinated with PRP-D vaccine than among 398, previously studied, unvaccinated children (0% vs 3.5%). The children studied were healthy 3-year-olds, from whom throat swabs were taken during a well-child visit to their local health center. However, the control cultures were obtained before the nationwide vaccination study; thus temporal factors other than vaccination may have influenced the results. Geographic factors necessitated sending swabs by mail to the center of sample processing, which may have limited the sensitivity of the microbiologic assay. Additionally, possible exposure to the bacterium from contacts was not measured.

In child care centers in Dallas, a prospective study was done to determine the prevalence of Hib colonization between 1987 and 1989 (41). During this period, conjugate vaccination was introduced in the United States, so both vaccinated and unvaccinated children attended the centers. Of 283 children studied, 59 had received unconjugated polysaccharide vaccine, and 89 had received conjugate vaccine (of which 94% received PRP-D). The Hib acquisition rates over the surveillance period were 21% and 9%, respectively. Among children exposed to at least one child with a positive culture result, the efficacy of conjugate vaccination to prevent Hib colonization in an unmatched analysis was 64%. No effect on colonization was found with PRP vaccination.

A clinic-based study in metropolitan Atlanta found a decreased Hib carriage rate in a population of 2- to 5-year-old children, 75% of whom were vaccinated with an Hib conjugate vaccine (42), and in Apache and Navajo Indian children who had received the Hib-OMPC vaccine appropriate for their age (43). These studies were performed after vaccination programs had begun, when adequate control populations were not available, and logistic factors often necessitated suboptimal microbiologic procedures.

The district by district implementation of a conjugate vaccine (PRP-T) in the Oxford Region in England enabled examination of a conjugate vaccine's effects over time in contemporary groups of vaccinated and unvaccinated infants (44). The children were recruited at birth so as to minimize selection bias concerning Hib carriage. Sampling error was limited, as one person took all the throat swabs, and laboratory analysis was standardized and took place in a single center. Other factors that could have influenced exposure to Hib or susceptibility to carriage were subjected to statistical control. Infants who had received Hib conjugate vaccine at the ages of 2, 3, and 4 months had significantly lower Hib acquisition rates than controls. In accordance with this, the point prevalence of Hib carriage was consistently lower in vaccines than in controls at 6, 9, and 12 months of age. The rates of acquisition and the period prevalence of *H. influenzae* serotypes e and f did not differ between vaccines and controls.

How Does Conjugate Vaccine Against Hib Affect Carriage?

The unconjugated Hib polysaccharide vaccine raises the IgA antibody concentration in nasal secretions and saliva of both adults and children (45). However, it does not affect the oropharyngeal carriage of Hib (31). The same lack of effect on pharyngeal carriage has been seen with other parenterally administered polysaccharide vaccines: meningococcal group A and C and several serotypes in the *Streptococcus pneumoniae* polysaccharide vaccine (47,48).

Conjugate vaccines induce higher concentrations of serum anti-PRP antibodies in young children than polysaccharide vaccines. It has been suggested that high serum concentrations might lead to passive transudation of IgG antibodies to mucosal surfaces (49). In an infant rat model, the serum IgG antibody concentration needed for an effect on Hib

colonization was 7 µg/mL (50). More recent research has found that the presence and concentration of IgG anti-PRP antibodies in saliva correlated with the concentration in serum after conjugate vaccination (51). Secretory IgA anti-PRP antibody was found in the saliva of children who had no similar, detectable serum IgA. This suggests that the anti-PRP IgG in saliva was derived from serum, whereas the IgA antibodies were locally produced. It seems that conjugate vaccines can induce a mucosal IgA response as well as a systemic IgG response, and while there is some evidence that high serum levels of IgG are relevant to the prevention of acquisition of carriage, the role of IgA remains unclear.

A decline in the serum, and thus perhaps mucosal, anti-PRP antibody concentration after primary immunization offers a simple explanation for the time-dependent effect of conjugate vaccines on acquisition of Hib. The geometric mean titer (GMT) of serum anti-PRP antibody in children in Oxfordshire, at 5 months of age, soon after completing a course of Hib immunization, was 5 µg/mL (52). The GMT in these children at 12 months of age was 0.83 µg/mL (53). A concentration of 1µg/mL after immunization with Hib polysaccharide vaccine has been accepted as the concentration associated with long term protection against invasive Hib disease (54). Acquisition of Hib and prolonged Hib carriage may occur only below a threshold concentration of serum or mucosal antibody.

How specific antibody contributes to the demise, or inhibits the attachment of Hib in the pharyngeal mucosa remains unresolved. Possible mechanisms include antibody-mediated opsonophagocytosis, direct bactericidal activity, or stereotactic inhibition. However, simple antibody-mediated bactericidal mechanisms may not completely explain the modulation of colonization in vaccinated persons. Carriage was not rapidly curtailed when conjugate vaccine was administered to current Hib carriers (44). This result is not easily explained, although the intracellular sequestration of Hib (55) may render a source of organisms inaccessible to antibodies in secretions. It is also possible that conjugate vaccine does not add significant antigenic stimulus in a child carrying Hib, with Hib antigen present in the mucosa.

Vaccines against Hib intervene in the normal relationship between Hib and host by increasing serum and/or mucosal anti-PRP antibody concentrations in the host before the host has any exposure to the bacterium. One could speculate that the primary role of vaccines in limiting Hib colonization is to prevent the acquisition of organisms. Secondarily, a large boost in the antibody concentrations, caused by mucosal contact with Hib in an immunized host, could lead to the more rapid elimination of Hib from the mucosa. There is also tenuous evidence of decreased Hib colony counts in cultures from colonized, vaccinated children (28).

In ecologic terms, Hib may precariously occupy the pharynx. A small influence that upsets the ecology in vaccinated persons may have a profound effect on the population kinetics of Hib. A rise in the concentration of serum and mucosal anti-PRP antibodies in infants could be enough to dramatically affect the pattern of Hib transmission. It seems likely that this effect would initially be greatest in places of close contact between children: families and child care institutions. Subsequently, the effect would become evident in a more widespread population.

The paucity of data on the ecologic behavior of Hib in the pharynx, the influence of conjugate vaccines on this behavior, and the importance of this influence, have prompted attempts at mathematical modeling to predict the population kinetics of Hib carriage and disease after conjugate vaccination. These models remain in their infancy but are awaited with interest.

Because they differ in biochemical composition and immunogenicity, the conjugate vaccines may vary in their long-term protective efficacy and effect on Hib carriage and transmission. Additionally, differing immunization regimens in populations may affect any generation of herd immunity. For example, administering a booster dose of conjugate Hib vaccine after the age of 12 months may prolong the presence of high concentrations of anti-PRP antibody in children, thereby enhancing the potential to limit transmission of Hib in the population.

In their relationship with the human host, *Neisseria meninigitidis*, *S. pneumoniae*, and Hib have numerous and close parallels. With the use of principles similar to those applied to Hib vaccines, conjugate vaccines against these other encapsulated pathogenic bacteria are now being developed and tested. The findings in studies of Hib may be a paradigm for the effect of conjugate vaccines on colonization by *N. meninigitidis* and *S. pneumoniae.* It will be important to examine this possibility closely as the vaccines become available.

The effect of conjugate vaccines on Hib carriage has been established in epidemiologic terms, but molecular knowledge about vaccination and mucosal immunity is limited. Much remains to be learned about the interaction between host and bacterium at the mucosal surface and about the contribution of conjugate vaccines to this complex relationship.

Dr. Barbour, a Rhodes Scholar from Western Australia, received a D.Phil. at Oxford University with work on the influence of conjugated Hib vaccine on the oropharyngeal carriage of Hib. She is currently training in clinical pediatrics at the John Radcliffe Hospital in Oxford.

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