Prevention of *Haemophilus influenzae* Type b Colonization by Vaccination: Correlation with Serum Anti-Capsular IgG Concentration

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Concentrations of serum anti-*Haemophilus influenzae* type b (anti-Hib) capsular polysaccharide (CPS) ≥0.15 and ≥1.0 μg/mL are widely used as surrogates for protection against invasive Hib disease. However, the relationship between serum anti-Hib CPS following immunization and protection against colonization is not known, making it difficult to evaluate new Hib vaccines or combination vaccines. In the Dominican Republic, nasopharyngeal swabs were collected from 546 9-month-old infants who had received Hib conjugate vaccine at ages 2, 4, and 6 months and from 600 unvaccinated infants of the same age. The prevalence of Hib colonization was lower among vaccinated infants than among unvaccinated infants (0.9% vs. 2.3%). Among vaccinated infants, protection against colonization was significantly correlated with anti-Hib CPS concentrations ≥5 μg/mL 1 month following the third dose of vaccine. These results suggest that the concentration of serum anti-Hib CPS needed for protection against colonization is greater than that needed for protection for invasive disease.

In the United States and in several European countries, the widespread use of *Haemophilus influenzae* type b (Hib) conjugate vaccines for infant immunization has led to reductions in transmission of Hib organisms and herd immunity, that is, a reduced risk of Hib disease in unimmunized populations [1]. While there is good evidence that Hib conjugate vaccines can prevent colonization [1], the mechanism for this protection is unclear. Hib vaccines are administered as a parenteral injection, and yet they provide protection at the mucosal surface of the oropharynx. While one might expect mucosal immunity to derive from mucosal IgA antibodies, some investigators have proposed that high concentrations of serum IgG antibodies are responsible for the protection against carriage [2, 3]. Although serologic surrogates for protection against invasive disease exist (0.15 and 1.0 μg of anti-Hib capsular polysaccharide [CPS]/mL of serum are generally recognized surrogates for short- and long-term protection against Hib invasive disease, respectively), no human data support similar thresholds for protection against carriage. The establishment of a serologic surrogate for protection against colonization would be useful for evaluation of the expected effects of various vaccine regimens against colonization and for licensure of new Hib-containing vaccines. To test the hypothesis that high levels of serum anti-Hib CPS following vaccination correlate with protection from colonization, we measured serum antibodies and collected nasopharyngeal (NP) swab specimens from 546 immunized and 600 nonimmunized infants.

Materials and Methods

**Study population.** A total of 1200 infants were studied: 600 who had received a Hib conjugate vaccine and 600 who had not been vaccinated. At age 2 months, 600 infants were enrolled in a randomized trial of the immunogenicity of various Hib conjugate vaccine regimens, including regimens that utilized full doses (10 μg of polyribosylribitol phosphate [PRP] antigen), and one-half or one-third of the usual dose of Hib conjugate vaccine [4]. All infants received PRP-T Hib vaccine (Pasteur-Merieux/Connaught, Lyon, France) at ages 2, 4, and 6 months. One-half of the infants received PRP-T combined in the same syringe with DTP (diphtheria-tetanus...
toxoids–pertussis vaccine; Pasteur-Merieux/Connaught), and one-half received PRP-T as a simultaneous but separate injection. Serum specimens were collected from vaccinated infants at ages 4, 6, and 7 months. NP swab specimens were collected at age 9 months.

In the Hib vaccine immunogenicity study, there was no placebo group. To obtain a comparison group of infants who had not received Hib conjugate vaccine, we enrolled a convenience sample of 600 infants of the same age and from the same vaccination center who had not participated in the Hib vaccine study. These infants were enrolled and swabbed at age 9 months, the age of routine measles vaccination. No serum specimens were collected from the unvaccinated children. All children were residents of Santo Domingo, Dominican Republic, and were enrolled from a single health center. All children were swabbed over a 3-month period between February and April 1999.

Laboratory methods. NP swab specimens were collected by inserting a calcium alginate–tipped flexible wire (Calgiswab; Hardwood Products, Guilford, ME) through the nares until the posterior pharynx was reached. The swab was then rotated for 1–2 s and withdrawn. Swabs were placed into a transport medium (consisting of skim milk, tryptone soya broth, glucose, and glycerol) and transported under refrigeration to the laboratory within 4 h, where the specimens were plated onto antiserum agar plates. Antiserum agar plates were prepared at the Centers for Disease Control and Prevention (CDC) in Atlanta, using Levinthal agar and a modification of the method of Michaels et al. [5]. Antiserum agar plates were incubated in a 5% CO2 environment at 35°C–37°C for 3 days. Colonies surrounded with a “halo” appearance were suspected Hib colonies. These colonies were then subcultured onto chocolate agar plates (BD Bioscience, Cockeysville, MD). Each suspected Hib colony was tested for X and V factor dependency and serotyped by slide agglutination.

All serum specimens were frozen at −70°C and transported on dry ice. Serum concentrations of anti-Hib CPS were determined by EIA at the CDC. All concentrations were determined by comparison with standard reference specimens [4].

Statistical methods. To determine the relationship between antibody concentrations and Hib colonization, we stratified infants on the basis of their antibody concentration at age 7 months and compared the prevalence of Hib colonization at age 9 months. Reverse cumulative distribution plots were used to show differences in the distributions of antibody concentrations between the 2 groups [6]. Colonization rates and other categorical data were compared by Fisher’s exact test. Continuous data were compared by Student’s t test, or where data did not meet the assumptions for parametric testing, by Kruskal-Wallis test. Data analysis was conducted using SAS software (version 6.12; SAS Institute, Cary, NC) and Epi Info version 6.04b (CDC).

Results

We obtained an NP swab specimen from 546 (91.0%) of the 600 vaccinated infants and from 600 unvaccinated infants of the same age who attended the same vaccination clinic. The infants who received Hib vaccine and those who did not were similar in terms of sex, household tobacco smoke exposure, household size, and access to water in the home (table 1). Infants who received Hib vaccine were more likely to have indoor toilet facilities, to have had a fever, or to have taken antibiotics in the 10 days before collection of the NP swab.

At age 9 months, the prevalence of Hib colonization was higher in the unvaccinated group (14 [2.3%] of 600 colonized) than in the vaccinated group (5 [0.9%] of 546 colonized). The estimated reduction in Hib colonization at age 9 months was 61% (95% confidence interval [CI], −8.2% to 85.8%). When the 53 participants who received antibiotics in the 10 days before the swab were excluded, the reduction was 73% (0.6% vs. 2.2%, 95% CI, 7.4%–92.4%).

Of the 546 vaccinated infants with NP swab specimens, 492 (90.1%) completed the vaccination regimen according to protocol and provided a serum specimen following the third dose of Hib conjugate vaccine. The geometric mean concentration of serum anti-Hib CPS in this group was 9.66 µg/mL (95% CI, 8.7–10.7). The reverse cumulative distribution showed that while none of the colonized infants had ≥5 µg/mL of anti-Hib CPS in serum at age 7 months, nearly 70% of the uncolonized infants achieved levels ≥5 µg/mL (figure 1). The 5 vaccinated infants who were colonized with Hib at age 9 months had 0.42, 1.18, 2.24, 3.35, and 4.66 µg/mL serum anti-Hib CPS following the third dose of Hib conjugate vaccine. Infants with ≥5 µg/mL of anti-Hib CPS in serum at age 7 months were significantly less likely to be colonized with Hib at age 9 months than infants with <5 µg/mL of serum antibody (0 of 343 colonized vs. 5 [3.4%] of 149 colonized; P < .001, Fisher’s exact test). The prevalence rate in immunized children with <5 µg/mL of anti-Hib CPS in serum at age 7 months was not significantly different from the prevalence in unimmunized children (3.4% vs. 2.3%; P = .56, Fisher’s exact test).

Discussion

In this study, high levels of serum anti-Hib CPS following vaccination correlated with short-term protection against Hib
colonization. Specifically, infants with concentrations $\geq 5 \mu g/mL$ of anti-Hib CPS in serum at age 7 months were significantly less likely to be colonized with Hib at age 9 months than vaccinated infants with concentrations $<5 \mu g/mL$. The prevalence of Hib carriage in immunized infants with serum anti-Hib CPS $<5 \mu g/mL$ (3.4%) was similar to that observed in unimmunized infants of the same age (2.3%). Four of the 5 colonized vaccinees had serum anti-Hib CPS concentrations $>1.0 \mu g/mL$ at age 7 months. These results may indicate that the concentration of serum antibody needed for prevention of mucosal colonization is substantially greater than that needed for prevention of invasive disease.

In this study, the unvaccinated infants were children who attended the same large vaccination clinic but who were not part of the original randomized trial of Hib conjugate vaccination. As such, they were not randomly assigned to unvaccinated status and may differ slightly from the vaccinated infants, as evidenced by a lower proportion having an indoor toilet facility. It is unclear whether these differences are related to the risk of Hib colonization. The observation that the prevalence of colonization was similar among unvaccinated infants and vaccinated infants with $<5 \mu g/mL$ of anti-Hib CPS suggests that the effect, if any, was likely to be small. The comparison of prevalence among vaccinees with varying antibody concentrations are unaffected by this limitation.

Parenterally administered polysaccharide-protein conjugate vaccines protect against mucosal infections, including oropharyngeal colonization, pneumonia, and shigellosis [1, 7–10]. Cohen et al. [10] showed that higher serum antibody concentrations following immunization correlated with protection against shigellosis, a mucosal infection. By use of an infant rat model of NP colonization, Kauppi et al. [3] demonstrated that Hib colonization could be prevented by high levels of anti-Hib CPS in serum. In their model, $\approx 7 \mu g/mL$ of anti-Hib CPS in serum was needed to prevent colonization.

This is the first study in humans to correlate antibody concentrations in serum following vaccination with the risk of colonization. In this study, $\approx 5 \mu g/mL$ of anti-Hib CPS in serum was necessary to provide protection for $\geq 2$ months. However, this threshold concentration should be interpreted with caution. Infants were bled 1 month following their third dose of Hib conjugate vaccine but not at the time of the swab sample. Thus, it is not clear what level of antibody is needed at the time of challenge to prevent colonization, only that levels $\leq 5 \mu g/mL$ at age 7 months were correlated with protection at a point 2 months later. It is possible that antibody concentrations may have continued to rise in some infants or that they may have started to fall in others and that the true antibody concentrations at age 9 months differed from those measured at age 7 months. Likewise, this study did not assess whether protection was observed for $\geq 2$ months, and thus the duration of the effect could not be assessed. Also, for economic and logistic reasons, this study did not measure the concentration of secretory IgA or IgG in saliva, and their relative role cannot be directly assessed.

With an ever increasing number of vaccine products, including combinations of Hib and other vaccines, serologic surrogates of protection are increasingly important. In general, regimens that require fewer injections (e.g., combination vaccines) or reduce the cost of the vaccination program are preferred so long as the new regimens are likely to provide similar protection against invasive disease. There is a continuing controversy over the importance of circulating antibody concentrations as predictors of protection against invasive disease or whether immunologic priming, even in the absence of high concentrations of circulating antibody, is sufficient [11]. The results of this
study suggest that the amount of antibody required for protection against oropharyngeal colonization may be substantially greater than the level thought to correlate with protection against invasive disease (0.15–1.0 μg/mL). It is quite possible then that antibody concentrations elicited by new combination products or alternative regimens may be above the level needed for protection against invasive disease but below that for protection against colonization. In these circumstances, the possible effects, or lack thereof, on transmission and herd immunity will also need to be considered. Ongoing surveillance, even in the face of a >99% reduction in the disease, will play an important role in assessing any unexpected effects on carriage and transmission [12]. Further research into the relationship between vaccine-induced immunity and prevention of carriage may help to address the issues for Hib, and in the future, pneumococcal and meningococcal conjugate vaccines.

Hib Vaccine Evaluation Team Members

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References