RESPONSE TO CONJUGATE HAEMOPHILUS INFLUENZAE B VACCINE AMONG INFANTS IN NIAMEY, NIGER

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Abstract. Despite near elimination of Haemophilus influenzae b (Hib) meningitis from several industrialized countries following introduction of conjugate Hib vaccines in infant immunization schedules, Hib remains a major cause of meningitis and pneumonia in resource-poor countries. In Niger, Hib causes nearly 200 cases of meningitis per 100,000 children < 1 year of age, and > 40% of cases are fatal. We evaluated the immunogenicity of Hib polysaccharide-tetanus toxoid conjugate vaccine (PRP-T) administered in the same syringe as diphtheria-tetanus-pertussis (DTP) vaccine among infants in Niger. Infants were randomized into group 1 (PRP-T at six, 10, and 14 weeks), group 2 (PRP-T at 10 and 14 weeks), or a control group (meningococcal A/C polysaccharide vaccine). By 14 weeks of age, all subjects in groups 1 and 2 had ≥ 0.15 μg/ml of anti-PRP antibody, and 82% versus 76% had ≥ 1.0 μg/ml of antibody (P = not significant). By nine months of age the proportion of infants with ≥ 0.15 and ≥ 1.0 μg/ml was group 1 = 97% and 76%; group 2 = 93% and 67%; controls = 10% and 2.6%. Four weeks after the first, second, and third doses of PRP-T, infants in group 1 showed geometric mean titers (GMTs) of 0.19, 3.97, and 6.09 μg/ml while infants in group 2 had GMTs of 2.40 and 4.41 μg/ml four weeks after the delayed first and second doses. Both PRP-T groups had significantly higher GMTs at 18 weeks and nine months of age than infants in the control group. The Hib PRP-T vaccine was immunogenic in infants in Niger. The strong response after PRP-T was evident by six months of age. The early peak of disease is typically limited to a few reference hospitals, and concern about epidemics of meningococcal meningitis may overshadow appreciation for the importance of specific causes of endemic meningitis.

Routine use of Haemophilus influenzae b (Hib) conjugate vaccines in infant immunization programs has led to near elimination of Hib meningitis from several industrialized countries. Although H. influenzae is a major cause of meningitis in developing countries and is associated with high case fatality and substantial neurologic sequelae, Hib vaccines are not routinely used in countries with limited resources. In countries within the meningitis belt of sub-Saharan Africa, the contribution of H. influenzae b to childhood meningitis may be unrecognized since microbiologic confirmation of meningitis cases is typically limited to a few reference hospitals, and concern about epidemics of meningococcal meningitis may overshadow appreciation for the importance of specific causes of endemic meningitis.

Review of data regarding bacterial meningitis in Niamey, Niger from 1981 to 1996 identified a minimal estimate for the incidence of meningitis due to H. influenzae of 195/100,000 children less than one year of age and 52/100,000 children less than five years of age. More than 80% of Hib meningitis cases among children less than five years of age occurred during the first year of life (Figure 1), with 36% evident by six months of age. The early peak of disease evident in Niamey is typical of other developing countries, and has important implications for design of suitable vaccination programs for these areas.

Although numerous studies in industrialized countries have demonstrated the immunogenicity, efficacy, and effectiveness of Hib conjugate vaccines against invasive Hib disease, relatively little information is available regarding the response to these vaccines in children in sub-Saharan Africa, where the presence of nutritional deficiencies, concurrent illnesses such as malaria, or socioeconomic conditions might lead to differences in vaccine-induced protection. Recent demonstration that Hib conjugate vaccines protected infants in the Gambia from severe pneumonia as well as Hib meningitis suggest the enormous impact on childhood morbidity that these vaccines may have in Africa, where acute respiratory infections are a leading cause of childhood deaths.

We determined the response among Nigerien infants to an Hib conjugate (tetanus toxoid) vaccine, PRP-T (Pasteur Mérieux Sérum et Vaccins, Lyon, France) administered in two different schedules in conjunction with routine vaccinations of the Expanded Program on Immunization (EPI). We compared a standard three-dose primary series with a delayed schedule to assess whether two doses of PRP-T administered with the second and third dose of diphtheria-tetanus-pertussis (DTP) vaccine provided comparable immunogenicity to the three-dose regimen, and could therefore offer a more economical way to protect young children in Africa. Since vaccine cost has been a limiting factor in adoption of Hib vaccines worldwide, these data have implications for other countries considering adoption of Hib vaccines where cost is currently a barrier.

METHODS

Site. The study was conducted between January and November 1995 at the Yantala Center for Maternal and Child Health (CSMI) in Niamey, Niger. Niamey (1995 estimated population = 550,000) is located in southern Niger, which lies within the meningitis belt of sub-Saharan Africa. The Yantala CSMI serves a population of approximately 100,000 residents of west Niamey and offers family planning, prenatal care, well-child visits, consultations for sick children, and provides vaccinations through the EPI. All phases of this study were integrated into routine EPI activities at the Center.

Subjects. Children between the ages of four and 12 weeks were recruited for the study upon presentation for routine
EPI administration of the first diphtheria-tetanus-whole cell DPT vaccination. Parents gave informed consent to permit their children to participate in the study.

**Study vaccine.** The study vaccine was a single lot of polylisobetyl phospho-tetanus toxoid (PRP-T) conjugate vaccine produced by Pasteur Mérieux Sérum and Vaccins prepared with 10 μg of polysaccharide capsular antigen conjugated to tetanus toxoid. Lycophilized PRP-T vaccine was reconstituted with DTP for administration in the same syringe. The study vaccine or DTP alone was administered subcutaneously in the right subcapsular fossa. Infants in the control group received serogroup A polysaccharide vaccine produced by Pasteur Mérieux Sérum and Vaccins.

**Expanded Program on Immunization vaccines.** The EPI vaccines were provided to all infants enrolled in the study according to Niger's immunization schedule. Infants recruited into the study had already received BCG and oral polio vaccine (Pasteur Mérieux Sérum and Vaccins). At birth. At ages six, 10, and 14 weeks, they received a dose of DTP vaccine (Pasteur Mérieux Sérum and Vaccins) vaccines were administered.

Children in group 3 served as controls. These infants received two doses of meningococcal A/C polysaccharide vaccine at six and 14 weeks of age. Infants in the control group were given three doses of PRP-T vaccine at the end of the study (age nine months), with one month intervals between vaccinations.

Blood was collected by heel, toe, or fingerstick before each vaccination for groups 1 and 2 at six, 10, 14, and 18 weeks, as well as at nine months of age. Subjects in the control group had blood collected at six weeks, 18 weeks, and nine months of age (Table 1).

**TABLE I**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age at each contact</th>
<th>Blood sample</th>
<th>Blood sample</th>
<th>Blood sample</th>
<th>Blood sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Hib</td>
<td>6 weeks (4-12 weeks)</td>
<td>1 dose</td>
<td>Blood sample</td>
<td>1 dose</td>
<td>Blood sample</td>
</tr>
<tr>
<td></td>
<td>10 weeks (8-16 weeks)</td>
<td>1 dose</td>
<td>Blood sample</td>
<td>1 dose</td>
<td>Blood sample</td>
</tr>
<tr>
<td>2: Hib</td>
<td>14 weeks (12-20 weeks)</td>
<td>1 dose</td>
<td>Blood sample</td>
<td>1 dose</td>
<td>Blood sample</td>
</tr>
<tr>
<td></td>
<td>15 weeks (16-24 weeks)</td>
<td>1 dose</td>
<td>Blood sample</td>
<td>1 dose</td>
<td>Blood sample</td>
</tr>
<tr>
<td>3: Mne + C (control)</td>
<td>9 months (8-9 months)</td>
<td>1 dose</td>
<td>Blood sample</td>
<td>1 dose</td>
<td>Blood sample</td>
</tr>
<tr>
<td>All groups</td>
<td>OPV/DTP</td>
<td>OPV/DTP</td>
<td>OPV/DTP</td>
<td>Yellow fever</td>
<td></td>
</tr>
</tbody>
</table>

**Study design.** Children enrolled in the study were randomized into one of three groups (Table 1). Group 1 received three doses of PRP-T at the same time as DTP injections, scheduled for six, 10, and 14 weeks of age. Group 2 also received three doses of PRP-T, but administered with the second and third DTP injections at 10 and 14 weeks of age, and the last dose at nine months of age, at the visit when measles and yellow fever vaccines were administered. Children in group 3 served as controls. These infants received two doses of meningococcal A/C polysaccharide vaccine at six and 14 weeks of age. Infants in the control group were given three doses of PRP-T vaccine at the end of the study (age nine months), with one month intervals between vaccinations.

Blood was collected by heel, toe, or fingerstick before each vaccination for groups 1 and 2 at six, 10, 14, and 18 weeks, as well as at nine months of age. Subjects in the control group had blood collected at six weeks, 18 weeks, and nine months of age (Table 1).

The study sample size was designed to permit follow-up of a minimum of 38 children per group.

**Reactogenicity.** Children in the study were observed in the clinic for 15 min after each vaccination. Local and systemic reactions were evaluated in a sample of children from groups 1 and 3 one day after the first vaccination. Evaluation was conducted by a physician working at Yantala CSMI who was blinded to the study group of the infant.

**Laboratory methods.** Antibody directed against the polysaccharide PRP was measured by the Farr-type radioimmunoassay (RIA) method and expressed in terms of micrograms per milliliter. The reference serum for these assays used human anti-Hib (CBER/FDA lot 1983; Office of Biological Reagents and References, Food and Drug Administration, Bethesda, MD) containing 70 μg/ml of antibody. Assays were performed at the Clinical Seroimmunology Laboratory of Pasteur Mérieux Sérum and Vaccins on coded specimens. The lower limit of antibody detectable by the assay was 0.07 μg/ml. The PRP-antibody concentrations of ≥ 0.15 μg/ml and ≥ 1.0 μg/ml are considered to reflect short-term and long-term protection, respectively.

**Statistical analysis.** The SAS software version 6.12 (SAS Institute, Cary, NC) was used for the analyses. Proportions of subjects in each study group that reached antibody concentrations ≥ 0.15 μg/ml and ≥ 1.0 μg/ml at each age were equal.
compared using the chi-square test or, where appropriate, Fisher's exact test. A mixed model analysis of variance (ANOVA) was used to examine differences in geometric mean serologic antibody concentrations among the vaccine and control groups. The vaccine and control groups and the serum bleed number accounted for the fixed effects; the random assignment of individuals to one of the three study groups constituted the random effect. The mixed ANOVA was performed taking into account the repeated measures design of this study and the inherent collinearity among bleeds within an individual. A resultant longitudinal data analysis was performed using the MIXED procedure in SAS. For a number of specimens, the lower and upper limit of antibody concentrations were not titrated precisely (i.e., the lower limit was < 0.07 μg/ml and the upper limit ranged from > 2.2 to > 22.0 μg/ml). An accelerated failure time model was used to calculate geometric mean concentrations and 95% confidence intervals using the LIFEREG procedure in SAS. This method adjusted for the left and right censoring of these data.

**Ethical approval.** This study was approved by the Ethics Committee of the government of Niger as well as by the Institutional Review Boards of the Centers for Disease Control and Prevention (Atlanta, GA) and the World Health Organization (Geneva, Switzerland).

**RESULTS**

A total of 180 children were recruited for the study (Group 1 = 59, Group 2 = 62, Group 3 = 59), 87 (48.3%) of whom were female. The mean age at each visit was recruitment = eight weeks (range = 4–12); second contact = 13 weeks (range = 9–22); third contact = 18 weeks (range = 13–29); fourth contact = 24 weeks (range = 18–38 weeks), and final contact = nine months. Of these, 151 (83.9%) children completed the study. Sufficient sera for RIA analysis were available for 165 subjects at enrollment, 134 (74%) at the fourth contact, and 119 (66%) at the nine-month visit.

**Adverse reactions.** No serious adverse reaction was identified in any study subject. There was no difference between the frequency of fever, vomiting, diarrhea, excessive crying, or refusal to feed between groups 1 and 3. Swelling at the point of injection was more common among subjects who received the Hib vaccine (4 of 19) than among those who received the meningococcal polysaccharide vaccine (1 of 30) (P = 0.07, by Fisher's exact test, two-tailed).

**Immune response.** Table 2 shows the geometric mean titers (GMTs) of anti-PRP antibody at baseline and subsequent visits for each study group. There was no difference between baseline anti-PRP antibody titers between children in the three groups. Children in groups 1 and 2 had significantly higher antibody levels than the control group at the 18-week and nine-month visit.

**Infants in group 1 showed increased antibody levels after each dose, with a peak GMT at 18 weeks of 6.09 μg/ml (95% confidence interval [CI] = 3.67, 10.09). Subjects in group 2 responded strongly to the first PRP-T dose administered at the 10-week visit, reaching a GMT of 2.40 (95% CI = 1.70, 3.39) four weeks later. Antibody titers at 18 weeks did not differ significantly between groups 1 and 2. By nine months of age, GMTs decreased to 2.14 in children who had received three doses of PRP-T and 1.67 in children who had received two doses. These results did not differ significantly.

The proportion of children in each study group with antibody levels ≥ 0.15 and ≥ 1.0 μg/ml is shown in Table 3. Prior to vaccination, children in the three groups did not differ in the proportion with antibody levels ≥ 0.15 or ≥ 1.0 μg/ml. As expected, a higher proportion of children in group 1 than group 2 had antibody levels ≥ 0.15 or ≥ 1.0 μg/ml at the 10-week visit, when only group 1 infants had previously received PRP-T vaccine. Four weeks after the second PRP-T injection in group 1 and after the first PRP-T injection in group 2, all subjects had antibody levels > 0.15 μg/ml, and a similar proportion (82% and 76%) had levels ≥ 1.0 μg/ml. The proportion of children in group 1 and group 2 with levels ≥ 0.15 or ≥ 1.0 μg/ml at subsequent visits did not differ significantly.

Additional analyses revealed no effect of the precise age at vaccination or the interval (in weeks) between vaccinations on subsequent PRP antibody levels. There were too few subjects with high baseline PRP antibody to assess whether maternally acquired antibody to PRP affected response to subsequent PRP-T vaccine.

**DISCUSSION**

The PRP-T Hib conjugate vaccine administered in the same syringe as the DTP vaccine in conjunction with the routine childhood immunization schedule was immunogenic in infants in Niger. Overall, 86% of the children achieved antibody levels ≥ 1.0 μg/ml four weeks after a primary series of either two or three doses of PRP-T, and 71% maintained this level by nine months of age; 99% of the infants had antibody levels ≥ 0.15 μg/ml four weeks after the second or third PRP-T immunization and 95% maintained this level at nine months of age. These data suggest that Hib
The PRP-T vaccine is likely to provide excellent protection against Hib disease when administered to young infants according to the EPI schedule. There was little difference in the antibody levels at 18 weeks and nine months of age between children who had received two versus three doses of PRP-T vaccine when the two-dose schedule was delayed to begin one month after the first dose of DTP vaccine. Antibody response to Hib vaccines has varied in different studies according to diverse factors, including age at immunization, type of conjugate vaccine, and whether the vaccine is administered in the same syringe as other antigens. Response to certain Hib conjugate vaccines varied in different populations, suggesting that factors such as nutrition, pre-existing antibody to Hib or other antigens, or genetic influences could potentially affect immune response.

<table>
<thead>
<tr>
<th>Group</th>
<th>6 weeks</th>
<th>10 weeks</th>
<th>14 weeks</th>
<th>18 weeks</th>
<th>9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17/54</td>
<td>26/47</td>
<td>50/50</td>
<td>43/44</td>
<td>39/44</td>
</tr>
<tr>
<td>2</td>
<td>15/54</td>
<td>4/54</td>
<td>37/57</td>
<td>45/54</td>
<td>40/43</td>
</tr>
<tr>
<td>3 (control)</td>
<td>20/55</td>
<td>9/48</td>
<td>8/36</td>
<td>4/39</td>
<td>1/39</td>
</tr>
</tbody>
</table>

*No significant difference between group 1 and group 2 in % ≥0.15 or ≥1.0 μg/ml at 14 weeks, 18 weeks, or nine months of age. P < 0.001 for difference between group 1 and group 3 at ages 18 weeks and nine months, and between group 2 and group 3 at ages 18 weeks and nine months. P < 0.001 for difference in % ≥0.15 μg/ml between group 1 and group 2 at 10 weeks and P = 0.05 for difference in % ≥1.0 μg/ml between group 1 and group 2 at age 10 weeks. = no data.
different vaccines for administration at the six- and 10-week visits, these data suggest that where this schedule is feasible, a reduced number of doses of PRP-T should offer comparable protection to infants at lower cost. An additional concern in considering the optimal number of Hib doses is the magnitude of Hib disease that occurs before the delayed schedule would provide protection, since if this is large, the cost savings of eliminating a dose may be outweighed by the benefits of a three-dose schedule that protects against early disease. Surveillance for Hib meningitis in Niamey during the period 1981–1996 revealed that only 40 (6.5%) of 611 cases of Hib meningitis among children < five years of age occurred in infants < 12 weeks of age; 17 (2.8%) occurred among infants between eight and 12 weeks of age, cases that would potentially be prevented only by the traditional three-dose schedule (assuming two weeks are needed for antibody production following vaccination with the conjugate vaccine). Despite the official EPI vaccination schedule of six, 10, and 14 weeks of age, children in this trial received routine and study vaccines somewhat later (8, 13, and 18 weeks of age). One potential risk of delaying the first dose of PRP-T is that children in certain areas may not receive their second DTP injection until much later in infancy. Consideration of local circumstances, such as the coverage and timeliness of infant immunizations, can help EPI program managers and policymakers determine whether a delayed, two-dose schedule of PRP-T is appropriate for their setting. Other approaches, such as neonatal or maternal immunization programs, may address the earliest cases of infant Hib disease in areas where these cases constitute an important burden.23

We did not evaluate response to a booster dose of Hib conjugate vaccine. Data from the United Kingdom, where no booster dose is used, suggest that in an industrialized country, the primary series is adequate to dramatically reduce Hib meningitis.22 The earlier onset of Hib disease in developing countries might make a booster dose even less important for these populations, since most disease, 84% in Niamey, occurs by one year of age. However, carriage of Hib is more common among children in developing countries.24 The impact of the infant vaccination series on rates of carriage and disease in older children is not clear, although vaccinating older children had substantial impact on disease rates in younger infants in industrialized countries.12 Because the EPI infrastructure for delivering vaccines in Africa focuses on children less than one year of age, proposing a vaccination schedule that requires a dose administered to older children is problematic. Since most Hib disease occurs before 12 months of age, and exposure to carriers is likely to boost vaccine-induced immunity among children as they age, the potential value of a booster dose of Hib vaccine in developing countries is unlikely to merit the cost and infrastructure development it would entail.

Infants included in this study were typical of those residing in Niger, and probably representative of children throughout sub-Saharan Africa, in terms of nutritional status and exposure to other endemic diseases such as malaria. Vaccine administered in this study, provided in the same syringe as DTP by EPI personnel administering routine immunizations, was well-tolerated and immunogenic. These data therefore extend the observations collected in the Gambian regarding the potential benefits of Hib vaccine in children in Africa and are probably applicable to other tropical, resource poor areas where Hib disease rates are high.25 These findings also suggest that two-dose schedules should be given serious consideration, particularly when the economic savings of limiting the series to two doses would be sufficient to prompt policymakers to introduce Hib vaccines into routine use.

The excellent immune response evident among Nigerien infants suggests that these vaccines could prevent substantial illness in this region. Although much attention is routinely given to meningococcal epidemics, surveillance in Niamey over a six-year period including a major epidemic (1994–1995) revealed that meningitis deaths due to Hib and pneumococcus exceeded those due to meningococcus. Until success is achieved with other approaches to reducing the cost of Hib vaccines, such as through research into economic production of entirely synthetic Hib vaccine or investigations of lower dose formulations,26 reducing the number of injections may be the best means available to permit the largest number of the world’s children the benefit of protection against H. influenzae b disease.

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