CONCISE COMMUNICATIONS

The Induction of Immunologic Memory after Vaccination with *Haemophilus influenzae* Type b Conjugate and Acellular Pertussis–Containing Diphtheria, Tetanus, and Pertussis Vaccine Combination

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The significance of reduced antibody responses to the *Haemophilus influenzae* type b (Hib) component of acellular pertussis–containing combination vaccines (DTaP-Hib) is unclear. A DTaP-Hib vaccine evaluated in infants vaccinated at ages 2, 3, and 4 months showed reduced anti-Hib polysaccharide IgG (geometric mean concentration [GMC], 1.23 µg/mL; 57%, >1.0 µg/mL). Polyribitolribosyl phosphate (PRP) and Hib conjugate (PRP-T) vaccine given as a booster during the second year of life was evaluated for the presence of immunological memory. After boosting, most children achieved anti-PRP IgG >1.0 µg/mL, although the GMC was higher with PRP-T (88.5 µg/mL) than with PRP vaccine (7.86 µg/mL, P < .001). The GMC of the PRP group was higher than anticipated for naive PRP recipients of the same age. PRP-specific IgG avidity was significantly higher after boosting than after priming, providing further evidence for the generation of memory. Despite reduced immunogenicity, DTaP-Hib combination vaccines appear to prime for immunologic memory.

An increasing number of countries are changing from whole cell pertussis (wP) to acellular pertussis (aP) vaccines because of reduced reactogenicity. However, unlike wP vaccine combinations, aP-containing diphtheria, tetanus, and *Haemophilus influenzae* type b conjugate (DTaP-Hib) combinations have shown reduced immunogenicity of the Hib component [1]. Although the significance of this for clinical protection is unclear, the US Food and Drug Administration (FDA) recommends that DTaP and Hib vaccines not be given as a combined injection to infants [2].

We conducted a phase II trial of a DTaP-Hib combination vaccine in infants and observed reduced responses to the Hib component. To help evaluate whether vaccinees had been primed for memory, children were randomized in their second year of life to receive either Hib conjugate vaccine or plain Hib polysaccharide polyribitolribosyl phosphate (PRP) vaccine.

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Received 28 December 1998; revised 29 March 1999; electronically published 9 July 1999.

Written informed consent was obtained from parents. The study was approved by the ethics committees in North and East Hertfordshire District, the Institute of Child Health, and Great Ormond Street Hospital for Children National Health Service Trust.

Financial support: Department of Health, London; SmithKline Beecham Biologicals.

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The Journal of Infectious Diseases 1999;180:538–41 © 1999 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/99/8002-0043$02.00

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PRP as an immunogen can be used to mimic natural exposure to Hib and, in young children, can help differentiate booster from primary responses. In addition to IgG levels, we measured the avidity of PRP-specific IgG because an increase can be indicative of the establishment of immunologic memory [3].

Materials and Methods

Study population. Infants eligible for primary immunizations at ages 2, 3, and 4 months with diphtheria-tetanus-pertussis (DTP), Hib, and oral polio vaccines were recruited between June 1996 and January 1997 from general practices in Hertfordshire. Contra-indications to further doses were as specified in the national UK guidelines.

Vaccines and immunization schedule. All study vaccines were manufactured by SmithKline Beecham Biologicals, Rixensart, Belgium. The lyophilized Hib tetanus toxoid conjugate vaccine (PRP-T, Hiberix) contained 10 µg of PRP covalently linked to a purified tetanus toxoid (30 µg). The DTaP vaccine (Infanrix) consisted of separately purified pertussis antigens: 25 µg of pertussis toxoid (PT), 25 µg of filamentous hemagglutinin (FHA), 8 µg of pertactin (PRN) with 10 Lf of tetanus toxoid (potency >40 IU/dose) and 25 Lf of diphtheria toxoid (potency specification >30 IU/dose; fiducial limits of batch used 25-56 IU, as determined by the National Institute for Biological Standards and Control, Potters Bar, UK) adsorbed to 0.5 mg of aluminum hydroxide. The PRP-T vaccine was reconstituted with the DTaP vaccine and given by intramuscular injection into the thigh, arm, or buttock.

After reduced Hib antibody responses to the primary immunization course, a booster dose of Hib vaccine was offered to all study participants. Infants were stratified into three groups ac-
cording to their postprimary Hib IgG response (<0.15, 0.15–1.0, or >1.0 µg/mL) and were randomized to receive either a single dose of unconjugated PRP vaccine (containing 10 µg of PRP, no adjuvant) or a fourth dose of PRP-T.

Blood samples were obtained before the first dose, 4–6 weeks after the third dose, and before and 4–6 weeks after the booster immunization. Sera were separated, frozen, and stored at −20°C until tested for antibody levels.

Serologic studies. Sera were tested for PRP IgG by a standardized ELISA protocol, as described elsewhere [4]. Antibody concentrations were derived from an international standard preparation and expressed in micrograms per milliliter (lower level of sensitivity, 0.15 µg/mL). PRP IgG avidity was measured by modification of an ELISA incorporating the chaotrope, ammonium thiocyanate [5]. One change to the assay was the use of Hib capsular oligosaccharide conjugated to human albumin (Wyeth Lederle Vaccines and Pediatrics, Rochester, NY) as the solid-phase antigen, in contrast to the PRP-conjugated poly-L-lysine used previously. This change, brought about by the difficulty in obtaining plain PRP, precluded comparison with previously published PRP avidity values because of the lower values obtained with the modified assay. Serum IgG to PT, FHA, and PRN were measured by ELISA, as described elsewhere [6], and expressed in US IgG titerages according to US pertussis reference sera (lot 3 for PT/FHA; lot 4 for PRN).

Statistical evaluation. Antibody levels and avidity indices were log-transformed, and differences in geometric means were compared by regression or Student’s t test. Differences in proportions were compared by χ² or Fisher’s exact test. The relationship between PRP IgG levels and antibody levels to the other vaccine components was measured using Spearman’s correlation coefficient. Partial correlations between log-transformed antibody levels were calculated, to identify the independent correlations between each pair of antibodies after accounting for their correlation with the other antibodies.

Results

Study subjects. In all, 149 infants (87 boys, 62 girls) were recruited, and 148 received 3 doses of DTaP-Hib vaccine (1 child withdrew from the study after leaving the district). Pre- and postimmunization blood samples were obtained from 144 children, of whom 122 received a booster immunization; pre- and postbooster blood samples were obtained from 120 children (61 PRP, 59 PRP-T). The median ages at first dose and booster immunizations were 8 weeks (range, 7–12) and 16 months (range, 12–21), with no differences between study groups. The median interval between the third dose and the blood sampling was 48 days (range, 28–117) and between the booster dose and the blood sampling, 30 days (range, 27–56).

Immunogenicity. After primary immunization, only 57% of infants achieved a PRP IgG titer of >1.0 µg/mL (table 1), the level considered indicative of long-term protection, compared with 93% of the historical controls given DTwP-Hib vaccines [7]. After boosting, all vaccinees achieved PRP IgG titers above the minimum protective level (0.15 µg/mL), although the proportion achieving titers >1 µg/mL and the geometric mean concentration (GMC) of PRP IgG were higher in those boosted with PRP-T than in those boosted with PRP (P = .003 and P < .001, respectively; table 1).

In both booster groups, PRP IgG avidity was significantly higher 1 month after boosting than 1 month after completing the primary immunization series (fold increase: PRP group, 2.03, 95% confidence interval [CI], 1.64–2.51; PRP-T group, 1.55, 95% CI, 1.27–1.88). Despite the lower GMCs achieved in the PRP group, the avidity index was higher than in those boosted with PRP-T (P < .001; table 1). This difference was still apparent when the results were stratified by PRP IgG levels after the third vaccination (P < .001 within each postthird vaccine group; table 2). Within each booster group, the avidity index was not significantly different between those with post-third vaccination levels below or >1 µg/mL (PRP group, P = .58; PRP-T group, P = .14). The magnitude of the booster response, as measured by fold difference between pre- and postbooster PRP IgG level, was correlated with the fold increase in avidity between postthird and postbooster sera for those boosted with PRP (r = .36, P = .009), but not for those boosted with PRP-T (r = .02, P = .91).

Infants with low PRP IgG levels after the third dose had lower antibody titers to all other vaccine antigens (table 2). After adjusting for this individual responsiveness, partial cor-

Table 1. The geometric mean concentration (GMC) of anti-polyribitolribosyl phosphate (PRP) IgG, the proportions of children with antibody titers <0.15 or >1.0 µg/mL, the number with a >4-fold increase in IgG after a booster, and the geometric mean avidity index at age 5 months after primary immunization with 3 doses of acellular pertussis-containing diphtheria, tetanus, and Haemophilus influenzae type b conjugate (DTaP-Hib) vaccine and immediately prior to and 1 month after a booster dose of Hib conjugate vaccine or plain PRP given at a mean age of 16 months.

<table>
<thead>
<tr>
<th>Time measured, group tested</th>
<th>Anti-PRP IgG levels mg/mL</th>
<th>Avidity index, geometric mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG GMC (95% CI)</td>
<td>&lt;0.15 µg/mL n (%)</td>
</tr>
<tr>
<td>After primary, all</td>
<td>145</td>
<td>1.23 (0.98–1.58)</td>
</tr>
<tr>
<td>Before booster, all</td>
<td>120</td>
<td>0.25 (0.21–0.30)</td>
</tr>
<tr>
<td>After booster</td>
<td>61</td>
<td>7.86 (5.30–11.70)</td>
</tr>
<tr>
<td>Conjugate</td>
<td>59</td>
<td>88.5 (64.4–121.5)</td>
</tr>
</tbody>
</table>

Note: CI, confidence interval; ND, not determined.
relation analysis revealed the strongest independent positive correlation between the Hib and tetanus antibody levels ($r = .23, P < .05$). Despite the lower than specified diphtheria potency, all vaccinees had antibody levels $>$0.05 IU/mL after the third vaccine dose.

**Discussion**

Recent studies describing the reduced immunogenicity of the Hib component in DTaP-Hib combination vaccines [1, 8, 9], together with an increasing awareness of the importance of the induction of immunologic memory for long-term protection against Hib disease, have highlighted the need for sensitive ways to assess immunologic memory. Analyses of the antibody levels achieved after plain PRP or Hib conjugate vaccine boosters in primed infants have been used for this purpose, but interpretation of data has been hampered by the ethical constraints of generating contemporary control data from unprimed age-matched children given a single dose of plain or conjugated PRP. Historical comparisons may be informative but require careful review of the literature. For example, in a recent study of children with reduced primary responses to a combination vaccine [9], a PRP IgG GMC of 9.02 μg/mL after a booster dose of a Hib conjugate vaccine was considered indicative of a memory response despite higher values in the literature for a single dose of the same vaccine in unprimed 15-month-old children [10].

Our study is the first to directly compare PRP IgG levels induced by plain PRP or conjugated Hib booster in recipients of DTaP-Hib and to use the measurement of PRP IgG avidity to aid interpretation of the results. PRP is a plain polysaccharide. It can stimulate memory cells but elicits poor responses from naive B cells in children <2 years old. Thus, it is better than the highly immunogenic conjugate vaccines for assessing the presence of immunologic memory. An antibody titer $>1$ μg/mL is rarely achieved by toddlers receiving plain PRP for the first time in the second year of life [11]. In our study, the GMC achieved by the group boosted with plain PRP (7.86 μg/mL) indicates that immunologic memory was established.

Antibody avidity increases over time and after boosting in persons successfully primed for a memory response and has been used as a surrogate marker for the induction of memory to the Hib component of the DTwP-Hib conjugate [5] and of the pneumococcal capsular polysaccharides contained in pneumococcal conjugate vaccines [12]. In this study, PRP IgG avidity was greater after boosting than after primary immunization, providing additional evidence of the successful generation of immunologic memory by the DTaP-Hib vaccine, even in those with poor postthird PRP vaccine IgG responses. Of interest, the postbooster avidity index in the 3 infants with postthird vaccination IgG PRP levels $<0.15$ μg/mL who received a PRP booster (0.081, 0.097, and 0.078 μg/mL, respectively) was higher than that observed after primary immunization, although their postbooster PRP IgG levels increased only modestly (0.38, 0.69, and 2.63 μg/mL, respectively). Thus, T cell help and the generation of immunologic memory may occur in the absence of a detectable primary antibody response.

Children with reduced PRP IgG responses also had significantly reduced antibody responses to all vaccine antigens, but most markedly to the tetanus component. This finding supports the assertion of Dagan et al. [13], that dominance of carrier-specific B cells (also known as epitopic suppression) is unlikely to be the major mechanism responsible for the reduced Hib response. The general correlation between antibody responses to all vaccine antigens is also seen with DTwP-Hib vaccines in UK infants (Miller E, personal communication) and may reflect individual variation in the degree of age-dependent maturation of the immune system.

The rationale for developing aP vaccines was to reduce reactogenicity. However, the benefit of an improved reactogenicity profile with DTaP-Hib vaccines needs to be balanced against the possible disadvantages of a reduced Hib response or an

**Table 2.** Geometric mean (GM) antibody responses (range) to polyribitolribosyl phosphate (PRP), diphtheria, tetanus, and pertussis components of combined acellular pertussis-containing diphtheria, tetanus, and *Haemophilus influenzae* type b conjugate (DTaP-Hib) vaccine, and Hib response to boosting stratified by postthird vaccination anti-PRP level.

<table>
<thead>
<tr>
<th>Anti-PRP GM concentration (n)</th>
<th>$\leq 1.0$ μg/mL</th>
<th>$&gt;1.0$ μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>After PRP boost</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>After conjugate boost</td>
<td>2.93 (1.89–4.53)</td>
<td>17.8 (11.2–28.4)</td>
</tr>
<tr>
<td>GM avidity index</td>
<td>54.7 (35.6–84.2)</td>
<td>156 (107–228)</td>
</tr>
<tr>
<td>After PRP boost</td>
<td>0.104 (0.077–0.141)</td>
<td>0.114 (0.092–0.141)</td>
</tr>
<tr>
<td>After conjugate boost</td>
<td>0.061 (0.048–0.078)</td>
<td>0.078 (0.064–0.095)</td>
</tr>
<tr>
<td>GM titer of IgG after third vaccination (n)</td>
<td>62</td>
<td>82</td>
</tr>
<tr>
<td>Diphtheria IU/mL</td>
<td>0.51 (0.43–0.59)</td>
<td>0.94 (0.82–1.06)</td>
</tr>
<tr>
<td>Tetanus IU/mL</td>
<td>0.61 (0.52–0.70)</td>
<td>1.35 (1.19–1.53)</td>
</tr>
<tr>
<td>Pertussis toxin U/mL</td>
<td>29.95 (25.97–34.58)</td>
<td>46.31 (40.96–52.35)</td>
</tr>
<tr>
<td>PRN U/mL</td>
<td>57.02 (45.61–71.26)</td>
<td>114.7 (96.41–136.45)</td>
</tr>
<tr>
<td>Filamentous hemagglutinin U/mL</td>
<td>79.4 (68.15–92.51)</td>
<td>93.1 (79.3–109.3)</td>
</tr>
</tbody>
</table>
increased number of injections if, as recommended by the FDA, such combinations are not used in infants. Our study suggests that DTaP-Hib combination vaccines adequately prime for memory despite reduced Hib responses. The protective capacity of such combinations has not been studied, but the PRP IgG titers achieved after primary immunization with DTaP-Hib vaccines were higher than those seen with PRP–diphtheria toxoid conjugate vaccines, which have been highly effective in Finland [14]. In populations at high risk of early onset Hib disease [15], DTaP-Hib combination vaccines might not be appropriate, as the reduced levels of antibody in the period between priming and boosting may be important in determining protection. Avidity measures and booster responses are at best surrogates of protection, and careful postlicensure disease surveillance in countries switching to DTaP-Hib combination vaccines will be essential, particularly if no booster is given in the second year of life.

Acknowledgments

We thank the study nurses in Hertfordshire for carrying out recruitment and vaccination, Joan Vurdien and Teresa Gibbs for study administration, Pauline Waight for data analysis, Nick Andrews for statistical advice, Ted Ashworth for advice on the DTP antibody assays, and Maggie Vickers for help with the Hib avidity assays.

References