Safety and Immunogenicity of a Conjugate Vaccine against *Haemophilus influenzae* Type b in Splenectomized and Nonsplenectomized Patients with Cooley Anemia

Rolando Cimaz,1 Carolina Mensi,2 Emanuela D’Angelo,1 Elisabetta Fantola,1 Vittoria Milone,1 Luigi R. Biasio,1 Vittorio Carnelli,1 and Alessandro R. Zanetti2

1Istituti Clinici di Perfezionamento and 2Institute of Virology, University of Milan, Milan, and *Aventis Pasteur Merck Sharpe and Dohme, Rome, Italy*

Patients with thalassemia are at increased risk for infections, especially after undergoing splenectomy. Vaccinations and antimicrobial prophylaxis are recommended in these patients, but the optimal immunization schedule for *Haemophilus influenzae* type b (Hib) vaccine is unknown. The immunogenicity of a conjugate Hib vaccine was investigated in 57 patients with thalassemia, 32 of whom had undergone splenectomy. Anti–capsular antibodies to Hib (anti–polyribosylribitol phosphate) were measured before vaccination and 2, 6, 12, 24, and 36 months after vaccination. Immunization was well tolerated. All patients achieved protective (>1 μg/mL) antibody levels. Antibody titers declined after the initial postvaccination increase, becoming undetectable in 4 patients and decreasing to concentrations of 0.15–1 μg/mL in another 2 patients when tested 2–3 years after vaccination. Hib conjugate vaccine is safe and immunogenic in patients with thalassemia major; however, additional studies are needed to assess the need and timing of booster vaccination to maintain long-term immunity.

Patients with thalassemia major (Cooley anemia) are at increased risk for infections [1]. Chronic anemia, multiple blood transfusions, desferioxamine therapy, increased iron deposits in internal organs, and tissue hypoxia all contribute to increased susceptibility for viral and bacterial infections. Moreover, patients who undergo splenectomy are at major risk for infections caused by encapsulated bacteria, such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib), which can cause meningitis, pneumonia, bacteraemia, and other severe invasive infections. Septis occurs in ∼5% of patients who undergo splenectomy, and the risk is much higher in patients who undergo splenectomy for hematologic diseases than for those who undergo splenectomy after trauma [2, 3]. In addition, patients without spleens have lower specific antibody response, impaired complement-dependent opsonization pathway, and phagocytosis of unopsonized particulate matter than do healthy subjects [4, 5].

Vaccines against Hib are available and should provide long-term protection in healthy subjects [6]; serum antibody levels correlate with protection from bacteraemic infections. Several studies described pneumococcal vaccine in patients who underwent splenectomy, but little information is available on Hib vaccines in such populations [7–11]. Because the duration of antibody response to Hib after vaccination in patients with thalassemia has not yet been established, we vaccinated a group of patients with thalassemia with a Hib vaccine and assessed serum antibody levels before vaccination and at regular intervals ≤3 years after vaccination.

Patients and Methods

**Patients.** We enrolled 57 patients with thalassemia major who were follow-up in our clinics and never were vaccinated: 30 females and 27 males 4–41 years old (median, 21 years). Thirty-two patients had undergone splenectomy. Most patients (53 of 57) had received transfusions with filtered red blood cell concentrates at regular intervals (mean, 15 transfusions/year).

**Vaccine.** A single dose of conjugate Hib PRP-T-vaccine (Act-Hib; Aventis Pasteur Merck Sharpe and Dohme) was administered intramuscularly in the deltoid region to all patients, concomitantly with a 23-valent *S. pneumoniae* vaccine. After administration, patients were observed for 1 h for detection of possible immediate reactions and were instructed to immediately report any adverse effect that might occur later to the physician.

**Biologic samples.** Blood samples (2–3 mL) were collected during pretransfusion routine laboratory tests from each patient before vaccination and at 2, 6, 12, 24, 36 months after vaccination for the detection of antibody levels to polyribosylribitol phosphate (PRP), the capsular polysaccharide of Hib.
Laboratory methods. Anti-PRP antibody concentrations were determined by means of a commercial ELISA kit (IMMUNOZYME Hib IgG; Immuno GMBH), according to the manufacturer’s instructions. In brief, the ELISA for the detection of PRP-specific IgG uses a solid-phase coated with PRP from Hib and a peroxidase-conjugated anti-human IgG antibody as a probe. The antibody concentration in the sample was measured by using a reference curve that included 5 internal calibrators expressed in micrograms per milliliter. Samples with an absorbance exceeding the most concentrated calibrator were diluted with a Hib incubation buffer (0.01 mol/L Tris/HCl [pH 7.4], containing detergent and protein stabilizer, and 0.005% merthiolate) and were retested. Levels of anti-PRP antibody >1 μg/mL and >0.15 μg/mL were considered to be correlates of long-term and short-term protection, respectively, against invasive Hib.

Statistical analysis. Antibody concentrations were expressed in geometric mean titers (GMTs), and statistical analyses were performed after log transforming the data. When antibody levels were undetectable, we assigned the arbitrary value of 0.05 for calculation. Comparisons of GMTs between subsets of patients were made by using the 2-tailed Student’s t test or analysis of covariance (a multiple linear regression) to adjust for prevaccination titers. \( P < .05 \) was considered to be significant.

Results

All patients achieved antibody levels >1 μg/mL 2 months after vaccination; follow-up results were available for 52 patients (91%) at 2 years and for 50 patients (88%) at 3 years after vaccination. Antibody GMTs before vaccination and after 2 months were 1.98 μg/mL (95% confidence interval [CI], 0.3–15.1) and 15.4 μg/mL (95% CI, 5.3–45.7), respectively. After the initial increase, antibody levels gradually waned over the 3-year study period: GMTs (95% CI) after 6, 12, 24, and 36 months were 11.3 (2.4–52.4), 9.7 (2.2–43.7), 5 (0.8–33.1), and 2.8 (0.1–58.9) μg/mL, respectively. Anti-PRP levels became undetectable in 4 patients and decreased to concentrations between 1 μg/mL and 0.15 μg/mL in 2 additional patients when tested 2–3 years after vaccination. Of these 6 patients, 3 had undergone splenectomy.

Seventeen patients had transient increases of antibody titers during the follow-up, which presumably was due to natural encounter with Hib. As shown in table 1, no differences in the GMTs of anti-PRP antibody were seen at different times of follow-up among patients who and who had not undergone splenectomy. In addition, no differences in GMTs were observed over time when patients were grouped by number of years of blood transfusions. The vaccine was well tolerated, with only minor side effects (fever or local infiltrate) that resolved spontaneously in 2 patients. No symptomatic infection was observed during the study period.

Discussion

Despite evidence that patients with thalassemia may have an impaired immune response, all our patients developed an effective antibody response to Hib vaccine, although the response was not long-term in some patients. Current recommendations for persons who have undergone splenectomy include antimicrobial prophylaxis and vaccination against S. pneumoniae and Hib. A booster for S. pneumoniae is recommended after 5 years for these patients, but there is no definite indication about the need and timing of booster vaccination against Hib.

The effect of splenectomy on immune responses to polysaccharide antigens is controversial—with evidence both for and against normal serum opsonic activity and antibody responses to immunization [9–11]. Patients without spleens may require a higher concentration of anticapsular antibody than do healthy persons, to achieve protection against invasive Hib disease [7]. In a recent study, levels of IgG-specific responses to polysaccharide vaccines were similar for patients who had undergone splenectomy and healthy control subjects, whereas asplenic subjects had lower IgM concentrations than the control subjects [10]. The impaired antigen-specific IgM antibody responses elicited after immunization in such patients may explain, in part, the conflicting data from studies that measured only total (IgG plus IgM) antibody concentrations [10]. In our study, patients who had undergone splenectomy did not respond differently from patients who had not undergone splenectomy, in terms of both postvaccination antibody increase and antibody persistence.

Both the small number of patients examined and the lack of a control group of healthy subjects preclude us from making definite conclusions; however, our results suggest that the conjugate Hib vaccine is safe and immunogenic when given to persons with thalassemia major. We also provide evidence that vaccine-induced anti-PRP antibody levels decline over time in patients with thalassemia and that a few patients have antibody concentrations below the putative protective threshold (0.15 μg/mL) 2–3 years after vaccination. It is not clear whether the apparent decline in protection in these patients may be accompanied by an increase in susceptibility to Hib disease.

In immunocompetent persons successfully vaccinated, the de-
crease of antibodies below the protective level does not necessarily mean the loss of immunity, since the T cell–dependent memory response elicited by the Hib conjugated vaccine may outlast the presence of circulating antibodies and provide effective protection. In fact, conjugation of PRP to a protein converts it from a T cell–independent to a T cell–dependent antigen with the capacity to induce immunologic memory. In this direction, several Hib conjugate vaccine efficacy trials have shown that the efficacy of the respective vaccines was >90%, although only 60%–80% of vaccine recipients had protective levels of anti-PRP antibody [13–15]. There is evidence that the clinical protection against Hib disease in vaccinated infants outlasts the decline in anti-PRP antibody levels, but there is concern that patients without spleens would be at risk for acquiring invasive Hib disease as antibody concentrations wane over time. Thus, although additional studies are needed to assess the duration of immunity in such patients, it seems prudent to give a booster dose of Hib vaccine every 5 years to sustain long-term protection.

References