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NOTES

Serum Antibody Response to Polysaccharides in Children with Recurrent Respiratory Tract Infections

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We evaluated children (15-months old and older) with recurrent upper respiratory tract infections and normal levels of immunoglobulins in serum for specific polysaccharide immunodeficiency using an enzyme-linked immunosorbent assay method. Results showed that of 12 patients vaccinated with Act-HIB vaccine, one did not develop specific antibodies to *Haemophilus influenzae* type b, demonstrating that such immunodeficiency is present in Costa Rican children.

Infections of the upper respiratory tract in children are a main reason for visits with pediatricians, and these patients show a high morbidity rate. In some of these children, such infections have a recurrent pattern (2). At present, there are no uniform criteria to define recurrent infection, but several repeated infections in a year should be considered to constitute recurrent infection (9).

In recent years, an immunodeficiency characterized by the selective inability to respond to polysaccharide antigens has been described (5, 6). This defect is suspected in patients who suffer from recurrent upper respiratory tract infections, especially those caused encapsulated bacteria, and who have normal serum immunoglobulin levels (9, 12). The absence of antibody response to bacterial polysaccharides, in the presence of a normal response to protein antigens, is a common characteristic of these patients (1).

Different laboratory methods that determine specific antibodies toward bacterial polysaccharide antigens have been developed (8, 13). However, immunoenzymatic techniques such as enzyme-linked immunosorbent assay (ELISA) are used most often due to their simplicity and sensitivity. As an alternative, special plastic surfaces for the covalent attachment of polysaccharides, or the conjugation of polysaccharides to protein carriers, have been utilized (10, 13).

In this investigation three ELISA methods were standardized to detect children with polysaccharide-specific immunodeficiency in a population of patients with recurrent respiratory tract infections.

Population. All children older than 15 months of age with recurrent respiratory tract infections who had been referred to the Immunology Outpatient Clinic at National Children's Hospital in San José, Costa Rica, between October 1998 and June 1999 were included. All patients had normal serum immunoglobulin concentrations. Recurrent infection was defined as follows: (i) recurrent otitis, three episodes in 6 months or four episodes in 1 year; (ii) sinusitis and/or recurrent bronchopneumonia, two episodes in 6 months or three episodes in 1 year or; (iii) recurrent rhinopharyngitis, four episodes in 6 months or eight episodes in 1 year. Children were excluded from the study if they had a primary immunodeficiency, a chronic pulmonary illness, or a structural congenital malformation. Once the patients were identified, parents were asked for written consent. The study was approved by the hospital's investigation committee.

Two blood samples were collected from each patient. The first sample was collected before the application of the Act-HIB vaccine (Pasteur-Mérieux), and the second was collected 4 to 6 weeks later. Both serum samples, pre- and postvaccination, were stored at −20°C until analysis.

ELISA. *Haemophilus influenzae* type b (Hib) vaccine conjugated to diphtheria toxoid (Hib TITER; Lederle Laboratories) was used as an antigen to coat ELISA plates (Immulon 2; Dynatech Laboratories). To determine the optimal minimum concentration of antigen for coating, the following concentrations were tested: 1.0, 0.5, 0.25, 0.12, 0.06, and 0.03 μg/100 μl/well. The antigen was diluted in coating buffer (Tris, 0.05 M; NaCl, 0.15 M; pH 9.0) and adsorbed onto the wells of microtiter plates during incubation at room temperature overnight. After rinsing the plates five times with this buffer, the wells were blocked with 1% bovine serum albumin (BSA) in FALC buffer (Tris, 0.05 M; NaCl, 0.15 M; $ZnCl_2$, 20 μM; $MgCl_2$, 1 mM; pH 7.4) for 30 min. Then, the plates were decanted and different dilutions (100 μl/well) of international reference anti-Hib sera (70 μg of anti-Hib/ml, lot 1983; Food and Drug Administration, Washington, D.C.) starting at 1:50 were added. Dilutions were prepared in FALC buffer containing 1% BSA.

After 2 h at room temperature, the plates were rinsed five times with FALC buffer; goat anti-human immunoglobulin G (IgG)–alkaline phosphatase, diluted 1:5,000 in FALC buffer–

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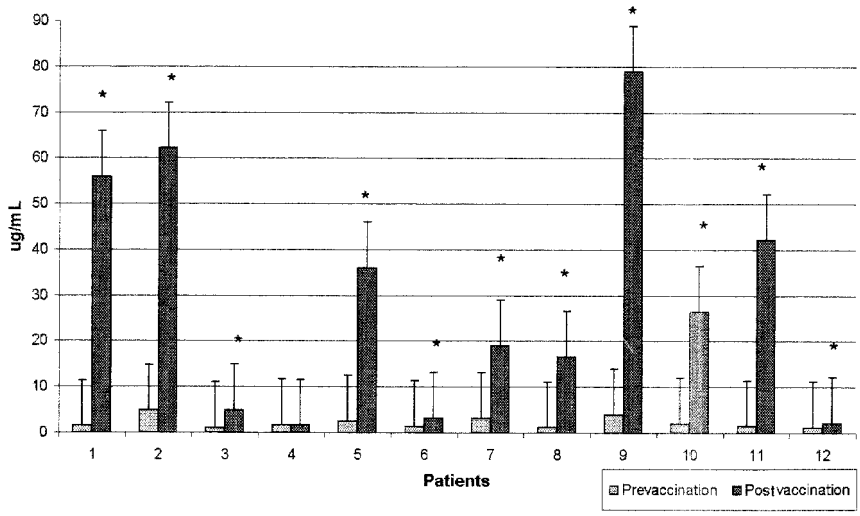

FIG. 1. Levels of anti-PRP IgG antibodies to Hib pre- and postvaccination, in serum of children with recurrent respiratory infection and normal serum immunoglobulin concentrations. Patient 1 had three doses and patient 4 had two doses of Act-HIB vaccine, prior to the study. Asterisks indicate significant ($P < 0.05$) increases from prevaccination levels.

1% BSA was added; and the plates were incubated for 2 h at room temperature. After washing, 100 μl of a 1-mg/ml concentration of *p*-nitrophenyl phosphate in substrate buffer was added to all wells. Absorbance readings at 410 nm were determined using a Dynatech MR5000 microplate reader. All samples were assayed in triplicate.

Pre- and postvaccination samples were analyzed at four different dilutions, with threefold serial dilutions starting at 1:100. Dilutions were prepared in FALC buffer–1% BSA. The background was established with wells without antigen, processed identically to a patient sera.

Hib vaccine (0.05 μg) conjugated to diphtheria toxoid was selected at an optimal concentration for coating microtiter plates, and a 1:300 dilution was selected as optimal for IgG antibody determinations.

An antibody response to polyribosyl ribitol phosphate (PRP) was considered positive if absorbance readings of the postvaccination serum sample were at least double the absorbance readings of the prevaccination sample when absorbance readings were higher than 0.1. For statistical comparisons, the Student *t* test was utilized. A *P* value of <0.05 was considered statistically significant.

During the 9-month study period, 12 patients were included, 8 male and 4 female, with ages between 15 and 44 months (median, 22 months). One patient had received three previous doses of Hib vaccine, and another one had been vaccinated twice. The rest of the patients had never been vaccinated against Hib.

Patient analysis. In this investigation, 12 children were evaluated. One patient did not show an IgG antibody response after vaccination (Fig. 1). Anti-diphtheria toxoid IgG antibody analyses demonstrated that postvaccine levels decreased or remained similar to prevaccine levels in 11 patients. In the one remaining patient, postvaccine antibody levels increased slightly (Fig. 2). All patients produced anti-tetanus toxoid IgG antibodies in high concentrations after immunization (Fig. 3).

This investigation evaluated polysaccharide-specific immunodeficiency in children showing recurrent upper respiratory tract infection and normal serum immunoglobulin concentrations. We evaluated 12 patients because to meet the criteria for selection for the study population, children needed to receive a booster vaccine and be at least 15 months old.

This population is not uniform because, at the time the study was done, the Costa Rican Social Security System did not provide Hib vaccination.

The inconvenience of using ELISA methods in the detection of antipolysaccharide antibodies lies in the difficulties encountered when these antigens are absorbed to the solid phase. Conjugation techniques sometimes result in alterations of the antigen's native structure (3–5). We utilized a commercial preparation of PRP conjugated to a carrier protein, which has been used for vaccination and which has shown good immunogenicity (9). This antigen was efficient in detecting anti-PRP IgG in children receiving an *H. influenzae* vaccine that was based on PRP conjugated to a different carrier protein. In spite of the small number of patients, it was proven that a specific

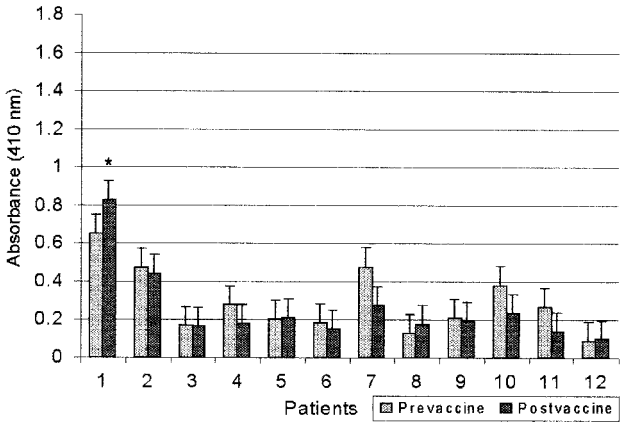

FIG. 2. Levels of anti-diphtheria toxoid IgG antibodies, pre- and postvaccination, in serum of children with recurrent respiratory infection and normal serum immunoglobulin concentrations, Asterisk indicates a significant ($P < 0.05$) increase from prevaccination level.

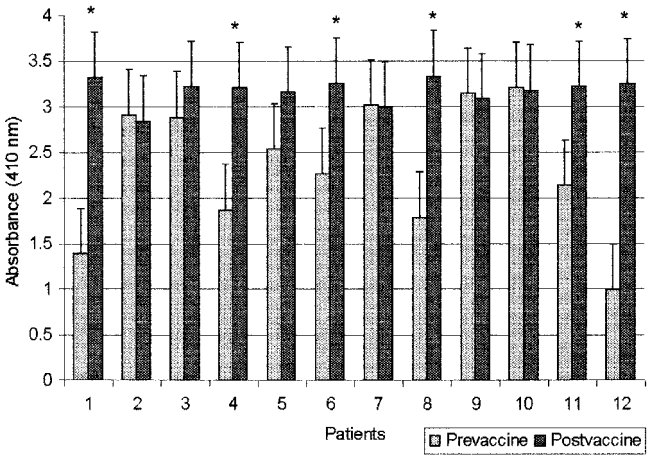

FIG. 3. Levels of anti-tetanus toxoid IgG antibodies, pre- and postvaccination, in serum of children with recurrent respiratory infection and normal serum immunoglobulin concentrations. Asterisks indicate significant ($P < 0.05$) increases from prevaccination levels.

immunodeficiency to polysaccharides of bacterial origin, detected by the absence of antibodies to polysaccharide antigens of bacterial origin, is a pathology present in Costa Rican children; thus, it may be a cause of recurrent infections of the upper respiratory tract in this population. The low percentage of detection of this specific immunodeficiency to the PRP polysaccharide of Hib is similar to that observed in countries such as Spain (7) and Brazil (11).

In addition, the present study verified that the tetanus protein can be used as an indicator of an adequate immune response to T-dependent antigens (1). This is because the minimal concentration present in the Hib TITER vaccine was sufficient to stimulate the immune system of the evaluated children without the need for vaccinating them separately with tetanus toxoid.

We did not check the patients' IgG subtypes before doing these ELISAs because a method was not available and clinical history does not coincide with children having an IgG subclass deficiency.

Since the causes of the specific immunodeficiency to the PRP polysaccharide of Hib are still unknown, we recommend that this study be repeated when these patients are more than 6 years old, in order to determine whether the cause of this immunodeficiency is retardation in the maturation of the immune system to polysaccharide antigens.

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