

Antibody response to unconjugated *Haemophilus influenzae b* and pneumococcal polysaccharide vaccines in children with recurrent infections

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Background: Increasingly, antibody testing is being used to evaluate the status of humoral immunity in patients with recurrent infection and suspected immunodeficiency. In the past, we had been impressed that immunization with unconjugated *Haemophilus influenzae b* (uHib) vaccine provided useful information about the ability to produce antibody to polysaccharides and that the use of pneumococcal polysaccharide (PPS) vaccine frequently produced results that were difficult to interpret.

Objective: The study was carried out to compare antibody responsiveness to vaccination with uHib with the response seen after PPS vaccination.

Methods: Twenty children (ages, 2 to 13 years; 11 male) who were referred to our immunology clinic because of recurrent infections were immunized with both uHib vaccine and PPS vaccine. Nine children had previously received conjugated Hib vaccine.

Results: All 20 children either responded with a twofold or greater increase in antibody titer after uHib vaccine or had preimmunization antibody concentrations of greater than 400 nanograms antibody nitrogen per milliliter (ng Ab N/ml). All of the children responded to PPS-3 with postimmunization antibody concentrations greater than 400 ng Ab N/ml. Three children had an increase in titer to PPS-7 of less than twofold, seven did not have a twofold increase in titer to PPS-9, and 15 had an increase in titer to PPS-14 of less than twofold.

Conclusion: Unconjugated Hib vaccine is a potent immunogen in children over 2 years of age. Prior immunization with the conjugate vaccine did not prevent a response to unconjugated vaccine. Unconjugated Hib vaccine appears to be at least as immunogenic as PPS-3 when used as an assessment vaccine for evaluating antibody responsiveness. (*J Allergy Clin Immunol* 1996;98:451-9.)

Key words: Unconjugated *H. influenzae b* vaccine, pneumococcal polysaccharide vaccine, recurrent infection, antibody response

The clinical immunologist is often called on to evaluate the patient with recurrent infection and suspected immunodeficiency. It is only rarely that a well-described immunodeficiency such as IgA deficiency, Wiskott-Aldrich syndrome, X-linked agammaglobulinemia, or common variable hypo-

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Received for publication June 29, 1995; revised Nov. 7, 1995; accepted for publication Nov. 13, 1995.

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0091-6749/96 \$5.00 + 0 1/1/70627

Abbreviations used

ARS:	Antibody response score
Hib:	<i>Haemophilus influenzae b</i>
ng Ab N/ml:	Nanograms antibody nitrogen per milliliter
PPS:	Pneumococcal polysaccharide
uHib:	Unconjugated <i>H. influenzae b</i>

gammaglobulinemia is diagnosed in such patients. If an immunologic defect is present, it is generally more subtle than that found in these well-characterized conditions. This observation has led to greater efforts to evaluate the immune system of

TABLE I. Patient characteristics

	Number evaluated	Range	Mean
Age		2-13 yr	88 mo
Gender	11 male: 9 female		
Principal recurrent infection			
Sinusitis	18/20 (90%)		
Otitis media	9/20 (45%)		
Pneumonia	3/20 (15%)		
Mastoiditis	2/20 (10%)		
Bacteremia	2/20 (10%)		
Total patients	17/20 (85%)		
undergoing surgery			
Sinus and/or ear surgery			
≥3+ Procedures	14/20 (70%)		
≤1 to 2 Procedures	3/20 (15%)		

patients with recurrent infection who do not have an obvious immunodeficiency syndrome.

One approach to evaluating these patients is the study of specific antibody responsiveness. There have been multiple reports of patients with an immunodeficiency characterized principally by an impaired antibody response to polysaccharide antigen.¹⁻⁸ Pneumococcal polysaccharide (PPS) vaccine is often used to evaluate antibody responsiveness in individuals with recurrent infection and suspected immunodeficiency. However, the response to PPS vaccine is often confusing because the patient may not respond to the various serotypes of the vaccine in a uniform fashion.^{9,10} Immunization with unconjugated *Haemophilus influenzae b* (uHib) can also provide useful information about the ability to produce antibody to polysaccharide antigen.^{5,6,11-14} In this study we compared the responses to both uHib and PPS vaccines in 20 children with a history of recurrent infection and suspected immunodeficiency.

METHODS

Patient population

This study was conducted over an 8-month period from July 1992 to March 1993 and involved 20 patients, ages 2 to 13 years, 11 of whom were boys. These patients were referred to the Immunology Clinic at LeBonheur Children's Medical Center because of recurrent infection and suspected immunodeficiency (Table I). For the purpose of this study, recurrent infection was defined as at least four episodes of sinusitis and/or otitis media in the previous 6 to 12 months, with concern on the part of the primary care provider great enough to warrant a referral to our immunology clinic.

TABLE II. Antibody response scores

Value	Definition
3	Hib ≥5000 ng Ab N/ml or PPS ≥2000 ng Ab N/ml before immunization
2	>Twofold increase with post titer ≥400 ng Ab N/ml; or <twofold increase, but pre value >400 ng Ab N/ml
1	>Twofold increase but post value <400 ng Ab N/ml, or <twofold increase with pre value ≤400 ng Ab N/ml and post value ≥400 ng Ab N/ml
0	<Twofold increase and post value <400 ng Ab N/ml

post, Postvaccination; pre, prevaccination.

Surgery to correct anatomic conditions that might contribute to recurrent upper airway infection had been performed in 17 children (Table I). Surgical procedures included sinus window surgery, endoscopic sinus surgery, tonsillectomy and adenoidectomy, placement of pressure equalization tubes, polypectomy, and septoplasty. Sixteen of the patients had undergone allergy testing.

Nine patients (45%) had previously received conjugated Hib vaccine. Three patients (15%) had received uHib vaccine only. Two patients (10%) were unsure whether they had received any type of Hib vaccine in the past, and six patients (30%) had not received any type of Hib vaccine.

Immunologic studies

Each of the patients in the study had, as a baseline evaluation, prevaccination *H. influenzae b* and pneumococcal antibody concentrations determined. Hib capsular polysaccharide polyribosylribitol phosphate antigen was used to measure Hib-specific IgG antibody by indirect enzyme immunoassay at Specialty Laboratories Inc. (Santa Monica, Calif.). An antibody concentration of greater than 100 nanograms antibody nitrogen per milliliter (ng Ab N/ml) is considered protective by that laboratory. Pneumococcal antibody levels were also measured by an indirect enzyme immunoassay (Specialty Laboratories, Inc.). Polysaccharide antigens of the *Streptococcus pneumoniae* serotypes commonly classified in the Danish system as 3, 7F, 9N, and 14 are used in this indirect enzyme immunoassay. A protective antibody titer is considered to be greater than 200 ng Ab N/ml. Nineteen unvaccinated normal control subjects (age range, 2 to 17 years; mean age, 92 months) had serum obtained for determination of PPS titers. Other serologic studies performed in most but not all patients included determination of quantitative serum immunoglobulins, isohemagglutinins, and tetanus antibody titers. Eleven patients had serum IgE quantitated, and an equal number had IgG subclass determinations.

Serum immunoglobulin (IgG, IgM, IgA) concentra-

TABLE III. Initial immune evaluations

	Number evaluated	Range	Mean
Immunoglobulins	19/20		
IgG		449-1440 mg/dl	919 mg/dl
IgA		8-240	120
IgM		56-211	124
IgE	11/20	4-140	44
IgG subclasses	11/20		
IgG ₁		305-1092 mg/dl	594 mg/dl
IgG ₂		25-291	108
IgG ₃		8-57	29
IgG ₄		7-195	49
Before PPS vaccination	20/20		
Serotype 3		45-3670 ng Ab/ml	956 ng Ab/ml
Serotype 7		10-250	85
Serotype 9		40-610	133
Serotype 14		50-1080	211
Before Hib vaccination	20/20	137-5001	1864

tions were determined by means of nephelometry (Behring, Marburg, Germany). Normal values were established by calculating an age-appropriate geometric mean for a group of normal children from the middle southern region of the United States. Levels of serum immunoglobulin were considered to be low if they were more than 2 standard deviations below this mean. IgE levels were calculated by using a radioimmunoassay. Serum IgG subclass isotypes were measured by Specialty Laboratories with an immunoradiometric assay. The age-adjusted normal ranges for this test were established by calculating a geometric mean from serum samples from more than 1000 children referred by 10 physicians from different regions of the United States. Values for our patients were considered low if they were greater than 2 standard deviations below the geometric mean for age.

Isohemagglutinin titers were measured in the LeBonheur Children's Medical Center hospital laboratory. For children between 24 and 36 months of age, titers greater than 1:2 dilutions are considered normal. For children over the age of 36 months, titers greater than 1:4 dilutions are considered normal. Tetanus toxoid antibody titers were determined by passive hemagglutination in our laboratory. A titer of 1:81 or greater is considered normal in a fully immunized child.

All of the patients evaluated were vaccinated with the uHib vaccine and with the PPS vaccine during the same clinic visit. Postvaccination antibody responses were obtained 3 to 6 weeks after immunization. Although antibody titers of greater than 100 ng Ab N/ml against *H. influenzae b* capsular polysaccharide and 200 ng Ab N/ml against a PPS capsular antigen are considered protective, in most normal subjects antibody titers in excess of these "protective" antibody concentrations will develop after immunization with the respective agents.^{6,7} Patients were considered poor responders or nonre-

sponders if postvaccination values were 400 ng Ab N/ml or less.

Vaccine

Unconjugated Hib vaccine was secured from the National Institutes of Allergy and Infectious Diseases, National Institutes of Health, *H. influenzae b*, Reference Laboratory at the University of Rochester, Rochester, New York. This vaccine is no longer commercially available because it has been replaced by vaccines consisting of *H. influenzae b* polysaccharide conjugated to a carrier protein. It is available for research use under Investigative New Drug 4093 on file with the Food and Drug Administration. A commercially available PPS vaccine (Pneumovax) was used.

Scoring of results

We established a scoring system for reporting the antibody responses (antibody response score [ARS]) to the uHib and PPS vaccines (Table II). This scoring system was based on a scale of 0 (poor to no response) to 3 (high protective levels before vaccination) and was used to help simplify data analysis.

RESULTS

Quantitative immunoglobulins, IgG subclass determinations, and nonpolysaccharide antibody studies

Nineteen children (95%) had quantitative immunoglobulins evaluated (Table III). Five of these had a low age-adjusted IgG value. Seven of 19 had an abnormal age-adjusted IgA value, but no absolute IgA deficiency was found. Eleven children had IgG subclasses evaluated. Three of these had a low IgG₃ value for age, and another three

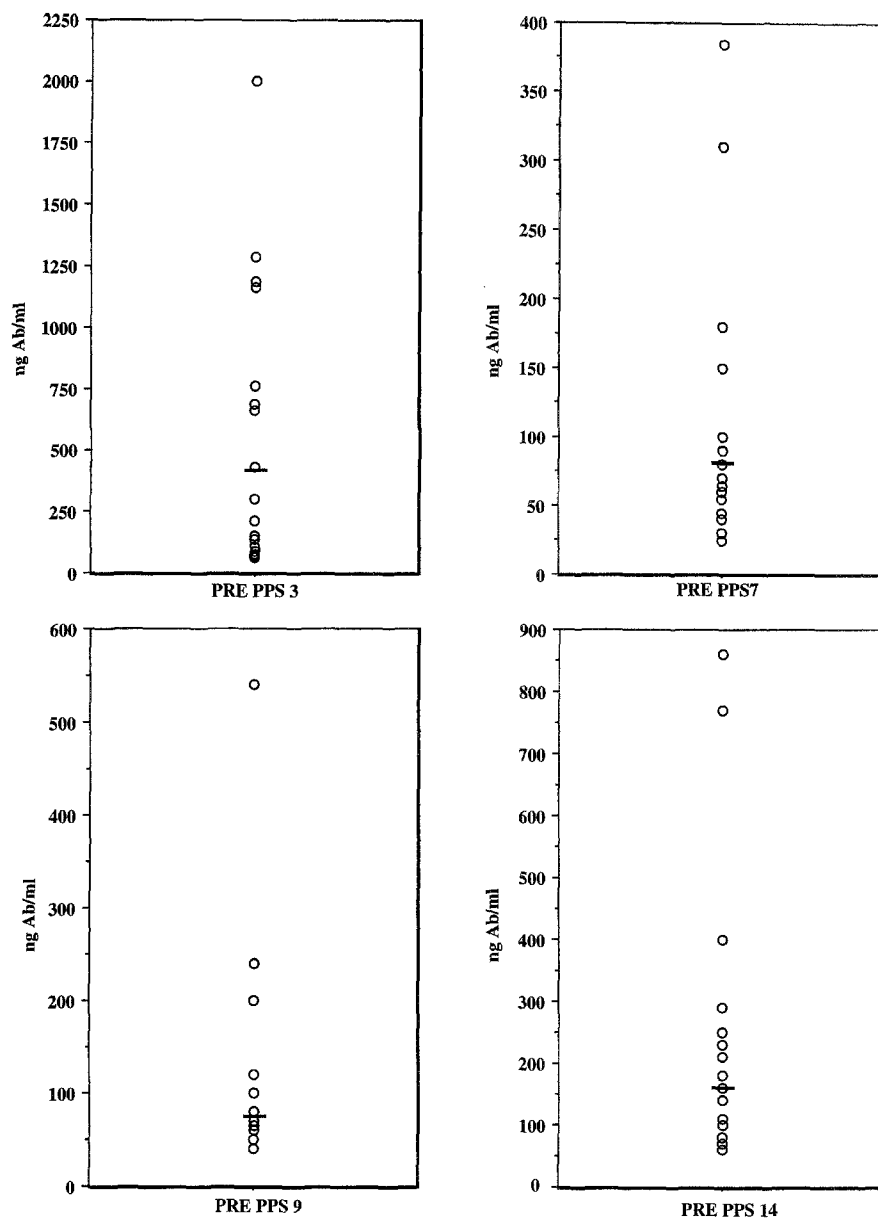


FIG. 1. Prevaccination responses to PPS serotypes 3, 7, 9, and 14 in 19 normal subjects. Some points represent more than one patient. Horizontal mark represents median.

had a low IgG₂ value for age. Fifteen children had tetanus titers evaluated. All had normal values. Nineteen children had isohemagglutinins studied. Only one of these had an abnormal value (blood type O with an anti-B titer of 1:1; anti-A titer was normal at 1:32). Of the 16 children who underwent allergy testing, seven had positive responses to at least one of the antigens studied.

Response to polysaccharides

Normal subjects. Antibody concentrations to the four serotypes in normal subjects are shown in Fig. 1.

Patients: PPS serotype 3. Nine children (45%) had prevaccination antibody concentrations greater than 400 ng Ab N/ml (Fig. 2, A). Five of these had concentrations greater than 2000 ng Ab N/ml. Of the 11 children with a prevaccination concentration less than 400 ng Ab N/ml, all had an increase of twofold or greater to PPS-3 after vaccination, and postvaccination titers were greater than 400 ng Ab N/ml. The mean ARS was 2.2 for serotype 3 (Table IV).

PPS Serotype 7. Only one of the children had a protective concentration of antibody to serotype 7 before vaccination (Fig. 2, B). Prevaccination an-

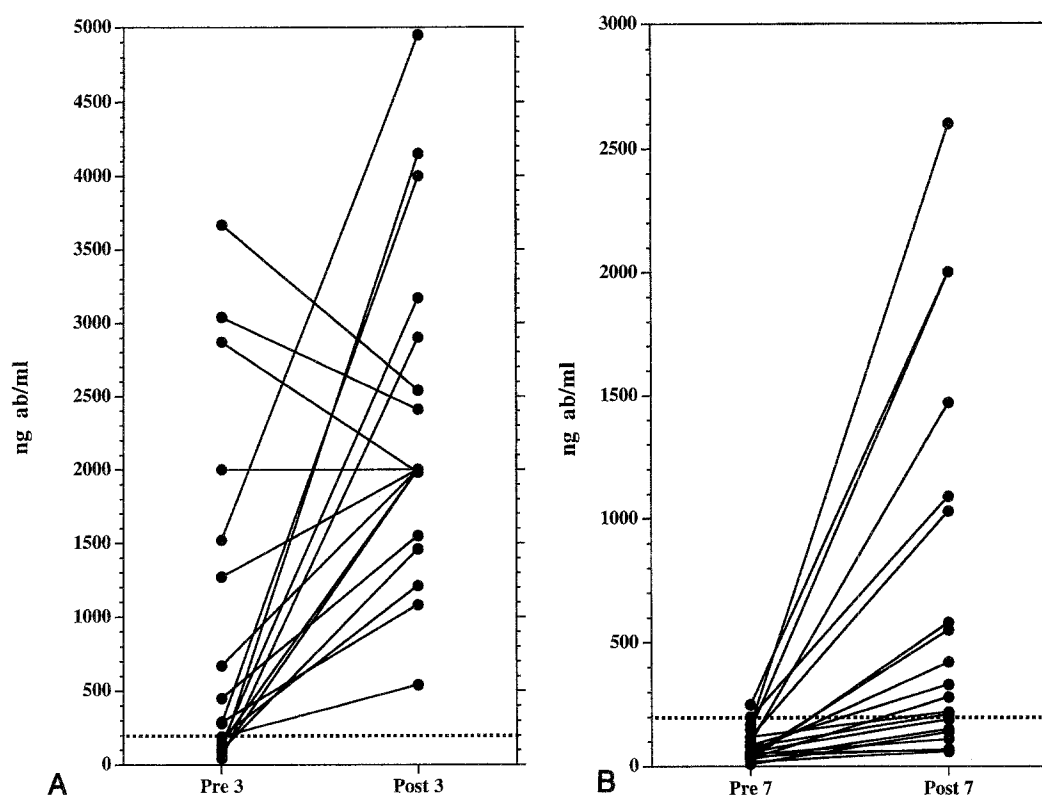


FIG. 2. A, Prevaccination and postvaccination responses to PPS-3. Twenty patients are represented. Some lines represent more than one patient. B, Prevaccination and postvaccination responses to PPS-7. Twenty patients are represented. Some lines represent more than one patient.

TABLE IV. Individual patient response scores

Patient No.	PPS-3	PPS-7	PPS-9	PPS-14	Hib
1	2	0	0	0	2
2	2	2	0	0	2
3	3	2	2	2	3
4	2	1	1	0	2
5	2	1	1	0	2
6	2	1	2	0	3
7	2	1	0	0	2
8	3	0	0	0	2
9	2	2	2	1	3
10	2	2	2	2	2
11	2	2	2	0	2
12	2	2	0	2	2
13	2	1	1	0	2
14	2	1	0	0	2
15	3	2	2	2	2
16	2	2	2	2	2
17	3	0	2	1	2
18	3	2	1	0	2
19	2	1	1	1	2
20	2	1	2	0	3
Average	2.2 ± 0.4	1.3 ± 0.7	1.2 ± 0.9	0.6 ± 0.9	2.2 ± 0.4
p Value	0.716*	<0.001	<0.001	<0.001	—

*When compared with Hib.

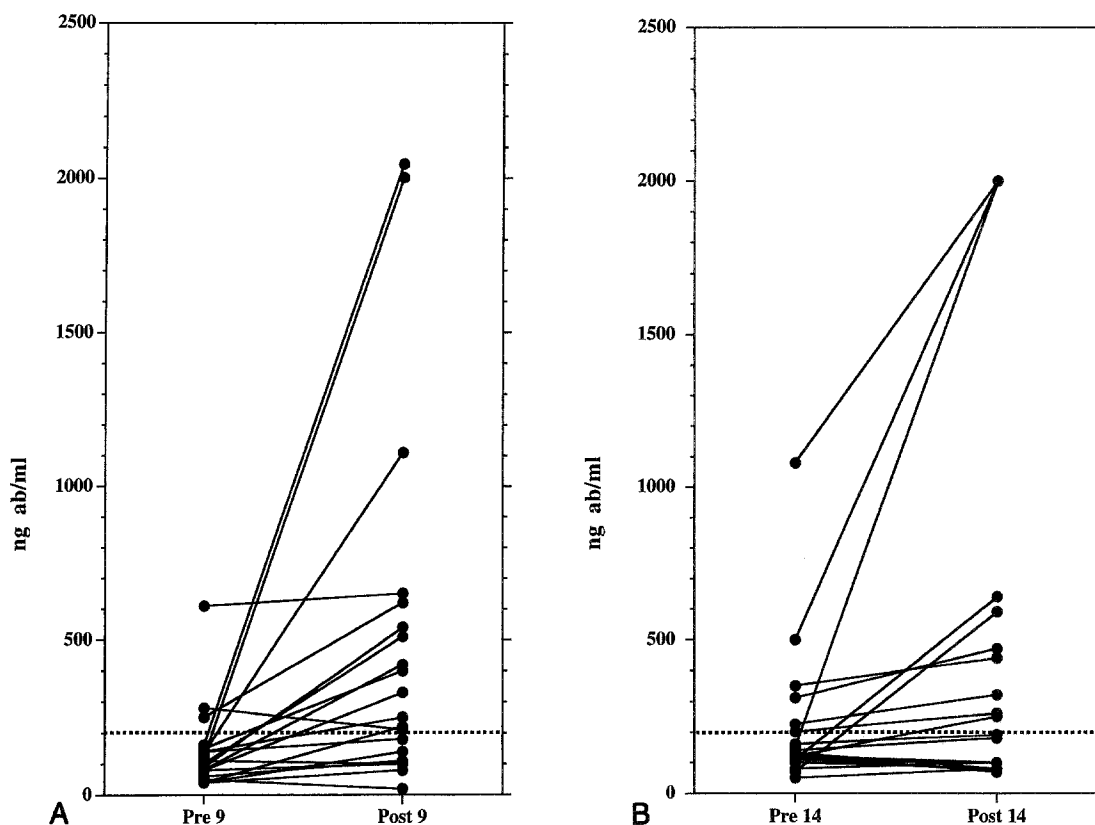


FIG. 3. **A**, Prevaccination and postvaccination responses to PPS-9. Twenty patients are represented. Some lines represent more than one patient. **B**, Prevaccination and postvaccination responses to PPS-14. Twenty patients are represented. Some lines represent more than one patient.

tibody concentrations ranged from 10 to 250 ng Ab N/ml. Nine children (45%) had an increase of twofold or greater to serotype 7 after vaccination, and postvaccination values were greater than 400 ng Ab N/ml (ARS of 2). Eight children (40%) had an increase of greater than twofold after vaccination, but postvaccination values remained less than 400 ng Ab N/ml (range, 60 to 330; ARS of 1). Three children (15%) had an increase of less than twofold and postvaccination levels less than 400 ng Ab N/ml (ARS of 0). The mean ARS was 1.3 for serotype 7 (Table IV).

PPS Serotype 9. One child (5%) had an antibody concentration of greater than 400 ng Ab N/ml before vaccination and did not exhibit an increase with vaccination (ARS of 2) (Fig. 3, A). Eight children (40%) had an increase of greater than twofold after vaccination with postvaccination values greater than 400 ng Ab N/ml (ARS of 2). Five children (25%) had an increase of greater than twofold after vaccination, but postvaccination levels were less than 400 ng Ab N/ml (range, 80 to 330; ARS of 1). Six children (30%) had an increase of less than twofold and post-vaccination levels less

than 400 ng Ab N/ml (ARS of 0). The mean ARS for serotype 9 was 1.2. (Table IV).

PPS Serotype 14. Two children (20%) had prevaccination levels greater than 400 ng Ab N/ml (ARS of 3) (Fig. 3, B). One of these also had a greater than twofold response to vaccination. Eighteen children (90%) had prevaccination values of less than 400 ng Ab N/ml. Three children (15%) had a greater than twofold response with postvaccination values greater than 400 ng Ab N/ml (ARS of 2). Two children (10%) had postvaccination levels greater than 400 ng Ab N/ml but an increase of less than twofold (ARS of 1). One child (5%) had a greater than twofold response but a postvaccination level of less than 400 ng Ab N/ml (ARS of 1). Twelve children (60%) had a less than twofold response and nonprotective postvaccination values (ARS of 0). The mean ARS for serotype 14 was 0.6 (Table IV).

Response to uHIB

Four children (20%) had prevaccination levels greater than 5000 ng Ab N/ml (ARS of 3) (Fig. 4). Thirteen children (65%) had prevaccination values

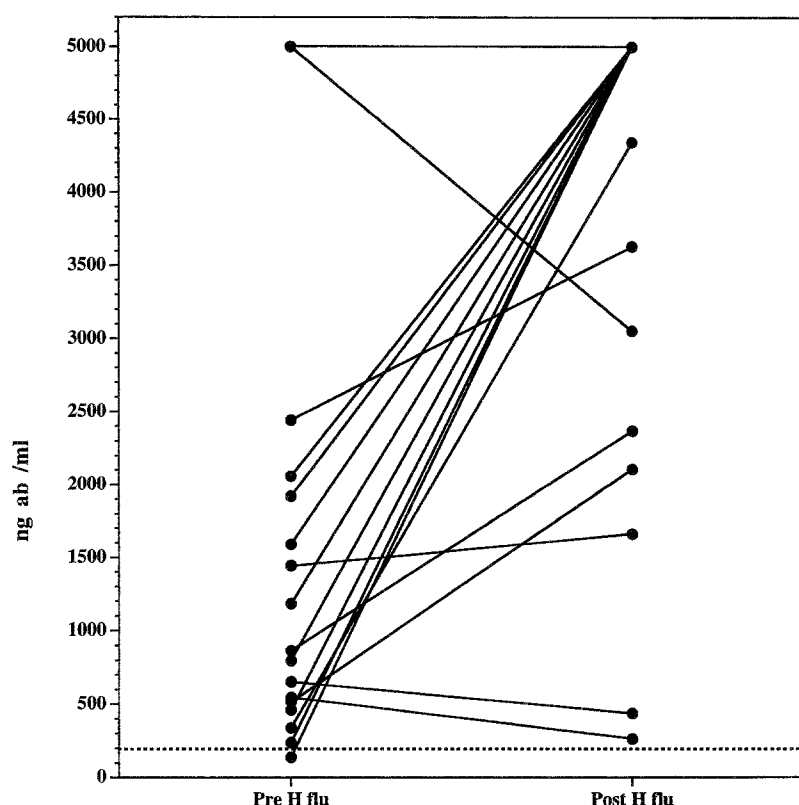


FIG. 4. Prevaccination and postvaccination responses to uHib. Twenty patients are represented. Some lines represent more than one patient.

between 400 and 5000 ng Ab N/ml (ARS of 2). Of these 13 children, eight showed an increase of greater than twofold with vaccination, two showed an increase that was less than twofold, and three showed no increase at all. Three children had prevaccination antibody levels less than 400 ng Ab N/ml, and all of these showed an increase of greater than twofold after vaccination and postvaccination values greater than 400 ng Ab N/ml (ARS of 2). The average ARS for Hib was 2.2 (Table IV).

Of the six children who had not received any type of Hib vaccine, one had a preimmunization level greater than 5000 ng Ab N/ml. Four of the six had an increase of greater than twofold with postimmunization values greater than 400 ng Ab N/ml. One of these children had no increase in antibody concentration after immunization but did have a preimmunization value greater than 400 ng Ab N/ml.

Two of the nine children who had received conjugated Hib in the past, had preimmunization levels greater than 5000 ng Ab N/ml. Three of the nine had an increase of greater than twofold with postimmunization levels greater than 400 ng Ab

N/ml, and four of nine had no increase but a prevaccination value greater than 400 ng Ab N/ml.

Of the three patients who had received unconjugated Hib in the past, one had a prevaccination antibody concentration greater than 5000 ng Ab N/ml, and two had an increase of greater than twofold with postimmunization values greater than 400 ng Ab N/ml.

Two normal unvaccinated children, ages 12 and 17 years, had Hib titers determined. These values were 318 ng Ab N/ml and greater than 5000 ng Ab N/ml, respectively.

Patient management

All patients were treated with prophylactic antibiotics. Each showed a decrease in the frequency of infection. Two of the patients were ultimately treated with intravenous immunoglobulin. Both responded well, with decreased frequency of infection.

DISCUSSION

Of the 20 children with recurrent infections whom we evaluated, all were considered to have

excellent antibody responses to PPS-3 and uHib. In each case, antibody levels were already sufficiently high to be protective, or they responded with a twofold or greater increase to protective levels. As has been observed in the past, response to the other PPS serotypes was somewhat ambiguous.^{8,9} The response to polysaccharides in human beings is normally not consistently seen until at least 2 years of age.¹⁴⁻¹⁶ Recognition of this stimulated the development of the conjugated form of the Hib vaccine.^{11, 12, 17-19} However, after the age of 2 years, immunologically normal patients should respond vigorously to polysaccharide antigen.

The pneumococcal vaccine is often used to evaluate the polysaccharide response. The significance of finding a good response to some but not all PPSs is unclear. One report has indicated that lower than expected responses to six or more of 13 serotypes is more likely to be associated with a history of recurrent infection.²⁰ Some serotypes, such as PPS serotypes 3 and 7, are more immunogenic than serotypes 9 and 14.^{21, 22} It is intriguing to speculate that the response to these less immunogenic serotypes might be useful as a more sensitive indicator of the responsiveness to PPS vaccination. However, we are unaware of any data to support this speculation.

Our data clearly demonstrate the potent immunogenic effect of PPS serotype 3. We had hypothesized that the response to uHib might represent a more sensitive means of examining polysaccharide responsiveness. The results of this study indicate that nothing is to be gained by adding uHib to an existing panel of vaccinations used to evaluate the immune response in children with recurrent infections, if such a panel already includes PPS antigens. The relatively high proportion (20%) of children with recurrent infection that we previously reported to be unresponsive to uHib can best be explained by noting that the majority of the poor responders in that study were around 2 years of age.⁶ This study demonstrates that children older than 2 years of age respond to uHib as well as they do to PPS serotype 3. Thus a poor response to either uHib or PPS-3 should alert the physician to a potential severe antibody deficiency.

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