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Haemophilus influenzae Type b Infection in Childhood: History of Bacteremia and Antigenemia

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Groups of children (mean age, 31.4 months) with *Haemophilus influenzae* type b meningitis, epiglottitis, or septic arthritis were tested for the presence and levels of bacteremia, capsular polyribophosphate (PRP) antigenemia, and development of specific antibody in serum after the onset of acute illness. Although bacteremia cleared promptly after antibiotic therapy, circulating PRP could be detected in serum for relatively long periods, with 51% of the patients still having detectable antigen after 30 days postinfection. Even in the presence of specific antibody, antigenemia persisted for as long as 47 days after admission. It was observed that there was no statistically significant correlation between the persistence of antigenemia and age ($P > 0.2$), the initial antigen concentration ($P > 0.50$), or the development of antibody ($P > 0.20$). The presence of a low magnitude of bacteremia (<300 organisms per ml) was associated with a maximum concentration of 10 ng of PRP per ml. On the other hand, bacterial counts in excess of 10^4/ml were associated with >1,000 ng of PRP per ml ($r = 0.98$, $r^2 = 0.96$, $P < 0.001$). It was observed that the amount of circulating PRP in the acute phase of illness was related to whether a child developed convalescent-phase antibody. Invariably, the younger children, who primarily had meningitis, had a PRP concentration of >10 ng/ml and failed to develop an antibody response in any isotype, whereas the older patients, who primarily had infections other than meningitis, had a PRP concentration of <10 ng/ml and a 45.5% success rate in developing an antibody response ($P = 0.006$). These findings suggest that there is a direct correlation between the magnitudes of bacteremia and antigenemia, that antigen may persist for long periods even in the presence of antibody, and that the level of antigenemia in addition to the patient age is significantly related to the nature of the convalescent-phase antibody response.

Haemophilus influenzae type b is the etiological agent of a wide spectrum of infections in children, yielding a significant degree of morbidity and mortality (2, 15). Limited information is available on the amount of capsular material (polymer of ribose, ribitol, and phosphate, or polyribophosphate [PRP] antigen) detected in patients' body fluids and the quantitative relationship to the number of organisms present. Furthermore, little is known about the persistence of this antigen in serum and its effect on the development of antibody in children. In the present study, the concentrations of PRP and viable bacteria were determined in the blood of patients with *H. influenzae* type b infections during the acute phase of illness, and the persistence of PRP from the time of admission to the last positive result was monitored. In addition, the antibody response was determined during both the acute and convalescent phases of illness.

MATERIALS AND METHODS

Study population and specimen processing. The patient population comprised 18 children, 10 males and 8 females, admitted during a 24-month period to The Children's Hospital of Buffalo with acute *H. influenzae* type b infections. It included 11 patients with meningitis, 5 with epiglottitis, and 2 with septic arthritis. The age of the patients at the onset of illness ranged from 5 months to 7.5 years, with a mean age of 31.4 months. The objectives and risks of the study were explained to the parents, and a signed statement of informed consent was obtained.

For all study patients, samples of blood were initially collected before the administration of antibiotic therapy, usually on the day of admission. Subsequent blood samples were collected up to day 89 postadmission. Serum was separated by centrifugation and stored at −20°C until tested. Blood cultures were processed as previously described (6–8). In brief, the blood was inoculated into both BACTEC aerobic bottles and sterile heparinized tubes for quantitative direct plating. A total of 1 to 3 ml of blood was inoculated into the bottles, and 0.1 to 1 ml was inoculated into the tubes. The time elapsed between the collection of the specimens and their arrival in the laboratory was usually 1 h or less.

Quantitation of viable *H. influenzae* type b in blood. The determination of the number of CFU of *H. influenzae* type b per milliliter in the blood was performed by a quantitative direct plating method as described elsewhere (6–8). Briefly, depending on the volume of blood obtained, a range of 0.05 to 0.5 ml of heparinized blood was directly pipetted onto sheep blood agar and chocolate agar plates and spread with a bacteriological loop for single-colony isolation. The plates were incubated at 37°C in 5 to 10% CO_2 and monitored for growth. The number of bacteria per milliliter of blood was calculated in relation to the size of the inoculum after 18 to 24 h of incubation. If the plates showed total confluent growth, the magnitude of bacteremia was taken to be >100,000 colonies per ml of blood. The determination of the CFU per milliliter in the cerebrospinal fluid from one patient with epiglottitis who subsequently developed meningitis has

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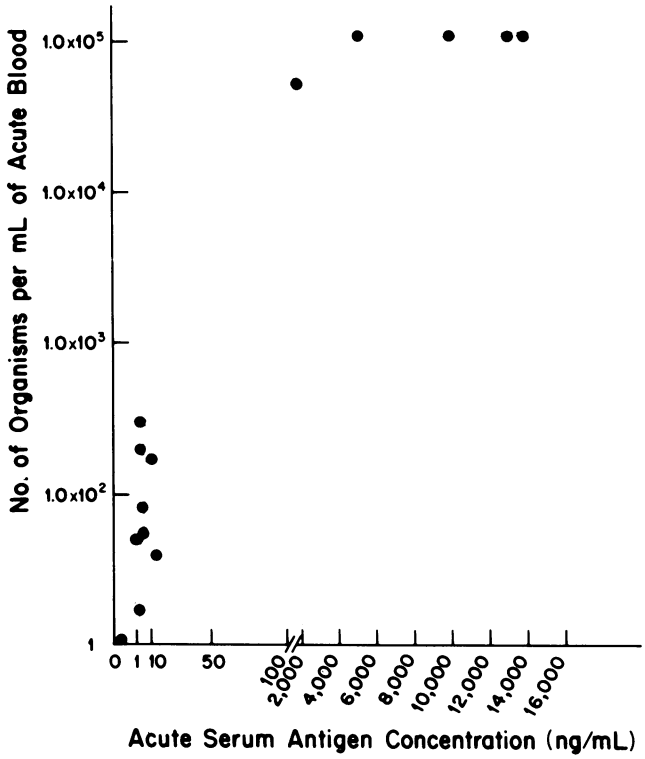

FIG. 1. Relationship of the number of viable organisms per ml of blood to the serum PRP concentration. Determinations with acute-phase blood samples were performed for 14 patients, 10 with meningitis and 4 with epiglottitis. $r = 0.98$; $r^2 = 0.96$; $P < 0.001$.

been previously described (6). The identification of all isolates was performed by conventional procedures.

***H. influenzae* type b capsular antigen assay.** PRP was detected and quantitated by an indirect enzyme-linked immunosorbent assay as previously described (11).

Serum antibody assay. The class-specific antibody responses in serum to *H. influenzae* type b were determined in 18 patients by a previously described enzyme-linked immunosorbent assay (11).

Statistical analysis. The relationship of the number of organisms per milliliter of blood to the serum antigen concentration was determined by using the Student *t* test after logarithmic transformation of the data. Correlation coefficients were determined for the persistence of serum PRP in relation to age, acute-phase antigen concentration, and convalescent-phase antibody. The relationship of the acute-phase PRP concentration to the convalescent-phase antibody response was determined by using the Fisher exact test.

RESULTS

Quantitative relationship of the number of organisms to the amount of PRP antigen detected. The relationship of the amount of *H. influenzae* type b PRP antigen detected in serum to the CFU per milliliter detected in blood is shown in Fig. 1. Determinations were performed with acute-phase blood samples from 14 patients, 10 with meningitis and 4 with epiglottitis. A significant correlation existed between the viable organisms detected and the antigen concentration ($r = 0.98$, $r^2 = 0.96$, $P < 0.001$). Essentially, 1.0 to 10.0 ng of PRP antigen per ml correlated with very low numbers of organisms (14 to 300). However, PRP concentrations of >1,000 ng/ml represented bacterial cell counts in excess of 10^4/ml.

Persistence of PRP in blood. For all patients the serum PRP concentration during the acute phase of the disease ranged from 0.13 to 13,847 ng/ml, whereas the level of antigenemia postinfection ranged from 236 to 0.10 ng/ml. The duration of detectable antigenemia for the whole patient group was 29.5 days, whereas for the children with meningitis, epiglottitis, and septic arthritis, it was 21.4, 23.3, and 86.5 days, respectively (Table 1). It is interesting that 51% of the patients still had detectable circulating PRP antigen after 30 days postinfection, with the remaining patients having detectable antigen for less than 1 month postinfection. Furthermore, three of the five (60%) patients positive for convalescent-phase antibody still had detectable antigen in serum after 30 days postinfection (Table 1).

Relationship of serum PRP persistence to age, initial antigen concentration, and antibody. To determine what factors influenced the persistence of PRP antigen in serum in children with *H. influenzae* type b infections, we evaluated age, acute-phase antigen concentration, and antibody. No significant correlations existed between PRP persistence and age ($P > 0.2$) or antigen concentration in the acute phase of the disease ($P > 0.50$) (Fig. 2). In acute-phase sera, no detectable *H. influenzae* type b-specific antibody activity was found, whereas 5 of 18 (28%) children developed antibody during convalescence. Of the five children who manifested an antibody response in all the serum immunoglobulin isotypes tested, four had epiglottitis and one had meningitis. In the presence of specific antibody, PRP antigenemia persisted in these five patients for 14, 24, 33, 38, and as long as 47 days after admission. However, no significant correlation existed between antigen persistence and convalescent-phase antibody titer ($P > 0.20$) (Fig. 2).

Relationship of acute-phase PRP concentration in serum to convalescent-phase antibody response. The serum PRP antigen concentration in the acute phase of illness was correlated with the development of a convalescent-phase antibody response in all isotypes (Table 2). Of the 18 children in the study, 11 had acute-phase serum PRP concentrations of <10 ng/ml (range, 0.13 to 7.5 ng/ml), and 7 had antigen concentrations of >10 ng/ml (range, 146 to 13,847 ng/ml). A significant correlation existed for 5 of the 11 (45.5%) children with acute-phase PRP concentrations of <10 ng/ml, who developed a convalescent-phase antibody response in all isotypes, in striking contrast to those patients with PRP concentrations of >10 ng/ml, who did not develop antibody in convalescence ($P = 0.006$) (Table 2). Of the five children who succeeded in developing an antibody response in convalescence, four had epiglottitis and one had meningitis; the mean age was 39.2 months. Of the 13 children who failed to

TABLE 1. Presence of PRP antigen and convalescent-phase antibody in the serum of 18 children with *H. influenzae* type b infections

Days postinfection	No. (%) of patients with detectable PRP	No. of patients with detectable convalescent-phase antibody
1–14	6 (33)	1
15–30	3 (16)	1
31–60	7 (39)	3
>60	2 (12)	0

develop antibody, 10 had meningitis, 1 had epiglottitis, and 2 had septic arthritis; the mean age was 22.2 months.

DISCUSSION

The findings summarized in this report indicate that in children with *H. influenzae* type b infections, the magnitude of PRP antigenemia in the acute phase of illness correlates both with age and with whether a child will develop convalescent-phase antibody. Furthermore, serum PRP antigen persists for surprisingly long periods and is not influenced by age, acute-phase antigen concentration, or antibody. In addition, there is a direct correlation between the magnitude of bacteremia and the concentration of serum PRP antigen.

It is of interest that circulating PRP persisted for relatively long periods, with 51% of the patients still having detectable antigen after 30 days postinfection. Even in the presence of specific antibody, antigenemia persisted for as long as 47 days after admission. It was observed that there was no statistically significant correlation between the persistence of antigenemia and age or between acute-phase antigen concentration and antibody. However, O'Reilly et al. (9) observed a significant inverse correlation between antigen persistence and antibody response. No significant correlation was found in the present study ($P > 0.20$), although the trend was similar.

Several studies with *H. influenzae*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Escherichia coli* attest to the fact that the concentration of antigen correlates with the severity of illness (1, 5, 10, 12). Related to this, a previous report (3) suggested that not only the amount of antigen but also antigen persistence may be a diagnostic indicator of slower recovery and more serious clinical disease. Our data, consistent with those of Granoff et al. (3), indicate that the persistence of antigen, often in high concentrations, is common. Furthermore, all patients in the present study recovered in the expected time periods, with no serious sequelae. The persistence of circulating PRP should not necessarily be interpreted as evidence of antimicrobial failure, since all blood cultures from our patients became negative shortly after the administration of antibiotics.

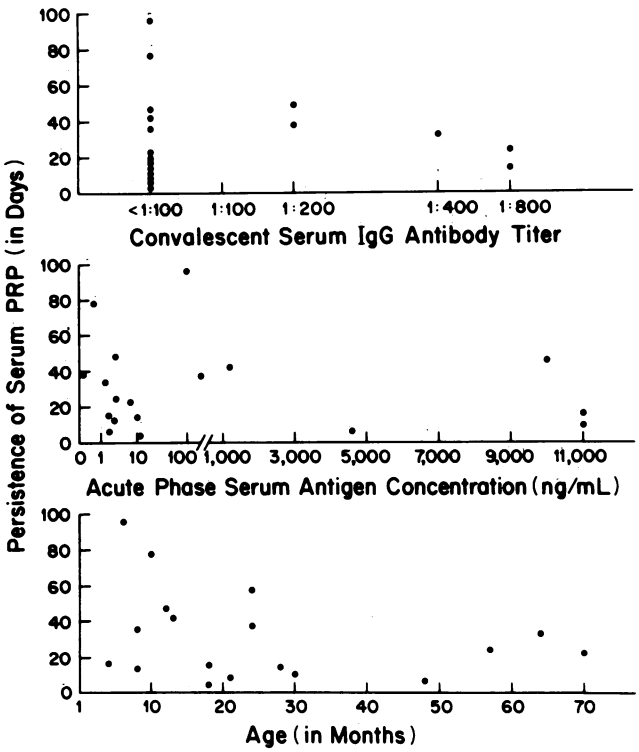

FIG. 2. Relationship of serum PRP persistence to age, acute-phase antigen concentration, and antibody. Determinations of serum PRP antigen and antibody were performed for 18 patients. In addition to the convalescent-phase serum IgG antibody titers represented, similar values were obtained for serum IgM and IgA.

TABLE 2. Relationship of the initial PRP antigen concentration to the development of the antibody response to PRP in 18 children with *H. influenzae* type b infections

Antibody isotype	Antibody response (geometric mean titer ± SD) in convalescent-phase sera at indicated acute-phase serum PRP concn	
	<10 ng/ml	>10 ng/ml
IgG	39.8 ± 1.8	0
IgM	30.2 ± 1.5	0
IgA	60.3 ± 1.4	0

An observation of particular importance in this study was that the amount of circulating PRP in the acute phase of illness correlated with the development of convalescent-phase antibody. Invariably, the younger children, who primarily had meningitis, had a PRP concentration of >10 ng/ml and failed to develop an antibody response in all isotypes, whereas the older patients, who primarily had infections other than meningitis, had a PRP concentration of <10 ng/ml and a 45.5% success rate in developing an antibody response. This observation is further supported by the results of O'Reilly et al. (9) indicating that antibody responses to *H. influenzae* uniformly were of a low magnitude in patients with prolonged antigenemia, irrespective of age. Furthermore, the results of another study (8a) indicated that subjects who exhibited an *H. influenzae* type b immunoglobulin G (IgG) antibody response in convalescent-phase serum samples had less than 100 CFU of the organisms per ml in the acute phase of the disease. On the other hand, subjects with high numbers of organisms (>1,000 CFU/ml) exhibited little or no antibody response in convalescent-phase serum samples (8a). Earlier results (11) consistent with these findings demonstrated that infants under 24 months of age with *H. influenzae* type b meningitis exhibited little or no serum *H. influenzae* type b-specific antibody response, although a nasopharyngeal IgA response was almost consistently detected. A recent report by Granoff et al. (4) indicated that the magnitude of the antibody response to *H. influenzae* type b polysaccharide-pertussis vaccine is related to the presence of the Km(1) allotype, providing further evidence suggesting genetic regulation of certain immune responses in humans. It should be emphasized that patient age is still a major determinant in whether a child will develop convalescent-phase antibody, as evidenced by the fact that the older children (mean age, 39.2 months) developed antibody, whereas the younger children (mean age, 22.2 months) failed to develop a response. Thus, it appears that in addition to the age and genetic factors, the magnitude of bacteremia and antigenemia in the acute phase of illness correlates with whether children with *H. influenzae* type b infections will produce convalescent-phase antibody. However, there is uncertainty about the causal relationships, since a circular

argument is present in that the age of the patient may directly affect the magnitude of antigenemia which, in turn, affects the antibody response.

Another interesting observation in this study was that essentially 1.0 to 10.0 ng of PRP antigen per ml detected by the enzyme-linked immunosorbent assay correlated with an upper limit of 300 cells and a lower limit of 14 cells. PRP concentrations of >1,000 ng/ml represented bacterial cell counts in excess of 10^4/ml. The variance in this relationship could be due to the fact that antigen detection measures both viable and nonviable cells, in contrast to just assaying CFU per milliliter. Furthermore, another factor could be strain variability in the amount of capsular material per cell. This degree of sensitivity of the enzyme-linked immunosorbent assay, in comparison with the results of previous reports (6, 13, 14), is much higher than that of counterimmunoelectrophoresis, microscopy, Gram staining, or acridine orange staining but similar to that of latex agglutination.

In conclusion, for children having *H. influenzae* type b infections, there is a direct correlation between the magnitude of bacteremia and that of antigenemia, and both of these factors, in addition to the age of the child, may play a role in whether a child will develop antibody during convalescence.

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LITERATURE CITED

1. **Coonrod, J. D., and M. W. Rytel.** 1973. Detection of type-specific pneumococcal antigens by counterimmunoelectrophoresis. II. Etiologic diagnosis of pneumococcal pneumonia. J. Lab. Clin. Med. **81:**778–786.
2. **Dajani, A. S., B. A. Asmar, and M. C. Thirumoorthi.** 1979. Systemic *H. influenzae* disease: an overview. J. Pediatr. **94:** 355–364.
3. **Granoff, D. M., B. Congeni, R. Baker, Jr., P. Ogra, and G. A. Nankervis.** 1977. Countercurrent immunoelectrophoresis in the diagnosis of *Haemophilus influenzae* type b infection. Am. J. Dis. Child. **131:**1357–1362.
4. **Granoff, D. M., J. P. Pandey, E. Boies, J. Squires, R. S. Munson, Jr., and B. Suarez.** 1984. Response to immunization with *Haemophilus influenzae* type b polysaccharide-pertussis vaccine and risk of *Haemophilus* meningitis in children with the Km(1) immunoglobulin allotype. J. Clin. Invest. **74:**1708–1714.
5. **Hoffman, T. A., and E. A. Edwards.** 1972. Group-specific polysaccharide antigen and humoral antibody response in disease due to *Neisseria meningitidis*. J. Infect. Dis. **126:**636–644.
6. **La Scolea, L. J., Jr., and D. Dryja.** 1984. Quantitation of bacteria in cerebrospinal fluid and blood of children with meningitis and its diagnostic significance. J. Clin. Microbiol. **19:**187–190.
7. **La Scolea, L. J., Jr., D. Dryja, and E. Neter.** 1981. Comparison of the quantitative direct plating method and the BACTEC procedure for rapid diagnosis of *Haemophilus influenzae* bacteremia in children. J. Clin. Microbiol. **14:**661–664.
8. **La Scolea, L. J., Jr., D. Dryja, T. D. Sullivan, L. Mosovich, N. Ellerstein, and E. Neter.** 1981. Diagnosis of bacteremia in children by quantitative direct plating and a radiometric procedure. J. Clin. Microbiol. **13:**478–482.

8a. **La Scolea, L. J., Jr., S. V. Rosales, R. C. Welliver, and P. L. Ogra.** 1985. Mechanisms underlying the development of meningitis or epiglottitis in children following *Haemophilus influenzae* type b bacteremia. J. Infect. Dis. **151:**1162–1165.

9. **O'Reilly, R. J., P. Anderson, D. L. Ingram, G. Peter, and D. H. Smith.** 1975. Circulating polyribophosphate in *Haemophilus influenzae* type b meningitis. J. Clin. Invest. **56:**1012–1022.
10. **Robbins, J. B., G. H. McCracken, Jr., E. C. Gotschlich, F. Orskov, I. Orskov, and L. A. Hanson.** 1974. *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. N. Engl. J. Med. **290:**1216–1220.
11. **Rosales, S. V., L. J. La Scolea, Jr., and P. L. Ogra.** 1984. Development of respiratory mucosal tolerance during *Haemophilus influenzae* type b infection in infancy. J. Immunol. **132:**1517–1521.
12. **Rytel, M. W.** 1975. Counterimmunoelectrophoresis in diagnosis of infectious disease. Hosp. Pract. **10:**75–82.
13. **Scheifele, D. W., R. S. Daum, V. P. Syriopoulou, G. R. Siber, and A. L. Smith.** 1979. Comparison of two antigen detection techniques in a primate model of *Haemophilus influenzae* type b infection. Infect. Immun. **26:**827–831.
14. **Shaw, E. D., R. J. Darker, W. E. Feldman, B. M. Gray, L. L. Pifer, and G. B. Scott.** 1982. Clinical studies of a new latex particle agglutination test for detection of *Haemophilus influenzae* type be polyribose phosphate antigen in serum, cerebrospinal fluid, and urine. J. Clin. Microbiol. **15:**1153–1156.
15. **Todd, J. K., and F. W. Bruhn.** 1975. Severe *H. influenzae* infections: spectrum of disease. Am. J. Dis. Child. **129:**607–611.