

## Occult Bacteremia with Nontypeable *Haemophilus influenzae*

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**A 2-year-old boy had occult bacteremia with nontypeable *Haemophilus influenzae* 6 weeks after receiving *H. influenzae* type b polysaccharide vaccine. Evaluation of his host defense was normal. As determined by outer membrane protein electrophoresis and Southern hybridization analysis, this strain was not related to type b strains. Its virulence in rats was similar to that of another nontypeable strain and less than that of a type b strain.**

*Haemophilus influenzae* type b is an important cause of invasive disease, such as meningitis and bacteremia, in infants and young children. In contrast, bacteremia due to nontypeable *H. influenzae* occurs much less frequently, and its occurrence is largely limited to adults with pulmonary disease (14) and to puerperal infections of women and newborns (13). Although it is an important etiologic agent in otitis media and sinusitis, it rarely causes bacteremia in young children. We report a 2-year-old boy who developed nontypeable *H. influenzae* bacteremia 6 weeks after immunization with *H. influenzae* type b vaccine.

A previously healthy, 25-month-old boy had an abrupt onset of fever and a generalized seizure. Physical examination was normal. Laboratory investigation showed a total leukocyte count of 22,000/mm<sup>3</sup> with 85% polymorphonuclear leukocytes, normal cerebrospinal fluid, and a normal chest radiograph. Blood culture yielded nontypeable *H. influenzae* susceptible to ampicillin, while urine and cerebrospinal fluid cultures were sterile. Ampicillin was given intravenously for a 7-day course with a clinical resolution. He had received *H. influenzae* type b vaccine (b-CAPSA I vaccine; Praxis Biologics, Rochester, N.Y.) 6 weeks previously. Further evaluation of his host defense showed no evidence of pulmonary, upper respiratory, or middle ear disease. He had normal serum immunoglobulin G (IgG), IgM, IgA, and IgG subclasses; C3, C4, and CH50; T and B lymphocyte numbers and subsets; and reduction of Nitro Blue Tetrazolium by his polymorphonuclear leukocytes. Serum antibody to *H. influenzae* type b capsular polysaccharide was detected at a concentration of 0.47 µg/ml (performed by radioimmunoassay at Praxis Biologics). Antitetanus antibody was detected. No Howell-Jolly bodies were seen on a Wright-stained peripheral blood smear. Nitro Blue Tetrazolium reduction by unstimulated and endotoxin-stimulated polymorphonuclear leukocytes was normal.

Identification of the isolate was confirmed by standard laboratory methods, including requirements for X and V factors for growth (6), by using the RAPID NH systems for biochemical identification of *Haemophilus* and *Neisseria* spp. (Innovative Diagnostics Systems, Atlanta, Ga.) and by the assessment of hemolysis on diphosphopyridine nucleotide-supplemented blood agar. Biotyping was performed by using the API 20E system (Analytab Products, Plainview, N.Y.) (6). Serotyping was performed by slide agglutination by using type-specific rabbit antiserum for capsular types a

through f (Centers for Disease Control, Atlanta, Ga., and Difco Laboratories, Detroit, Mich.), latex agglutination (for type b strains; Bactigen; Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N.J.), and counterimmunoelectrophoresis by using type-specific antisera for capsular types a through f (Burroughs Wellcome Co., Research Triangle Park, N.C.).

Preparation and polyacrylamide gel electrophoresis of outer membrane protein were performed by S. Barenkamp as previously described (1, 2). Southern hybridization analysis of chromosomal DNA from this strain was performed by S. Hoiseth as previously described (5).

To compare virulence, broth-grown, exponential-phase *H. influenzae* (10<sup>4</sup> CFU in 0.1 ml) was inoculated intraperitoneally into 5-day-old rats (Taconic Farms, Germantown, N.Y.) as previously described (12). In some experiments, cobra venom factor (purified from *Naja naja* venom; Cordis Laboratories, Miami, Fla.) at a dose of 300 U/kg of body weight was given 3 h prior to inoculation to deplete C3 (3). Blood (0.01 ml) was taken for culture following tail vein puncture 24 h after inoculation. Strain Eag is a laboratory-passed virulent type b strain (12). Strain Bon is a nontypeable *H. influenzae* strain isolated from the blood of a newborn with sepsis.

The blood culture isolate was confirmed as *H. influenzae*. The isolate was biotype 2, beta-lactamase negative, and susceptible to chloramphenicol and sulfamethoxazole-trimethoprim. It was nontypeable by slide agglutination and counterimmunoelectrophoresis. The colonial morphology was not iridescent on transparent (Levinthal) agar. (Colonies of encapsulated *H. influenzae* generally appear iridescent on this medium [6]). The profile of the outer membrane proteins as resolved following polyacrylamide gel electrophoresis was different from that of previously characterized type b strains (1, 2). Furthermore, Southern hybridization analysis of *EcoRI*-cut chromosomal DNA from this strain showed that it had no homology to a 9-kilobase DNA probe containing sequences necessary for type b capsule expression (5).

The virulence of the strain from the patient was evaluated by intraperitoneal inoculation of 5-day-old rats. None of five rats inoculated with this strain or with nontypeable strain Bon was bacteremic 24 h after inoculation, compared with five of five rats inoculated with *H. influenzae* type b Eag. When rats were pretreated with cobra venom factor, bacteremia was observed in two of five rats inoculated with the strain from the patient or strain Bon (mean bacterial densities, 10<sup>2.06</sup> and 10<sup>2.44</sup> CFU/ml of blood, respectively), while all five rats inoculated with type b strain Eag were dead (mean bacterial density, >10<sup>6</sup> CFU/ml of blood). There

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were no iridescent colonies recovered from the blood of animals inoculated with the strain from the patient.

Based on the microbiologic evaluation, the unsuccessful attempts to type the strain by using anticapsular antisera, the outer membrane protein electrophoresis profile, the absence of DNA fragments homologous to a cloned fragment of *H. influenzae* type b DNA (observed in all type b strains analyzed to date [5]), and the relative lack of virulence in an infant rat model of *H. influenzae* type b disease, the infecting strain was clearly a nontypeable *H. influenzae* strain. Although nontypeable *H. influenzae* strains which are genetically closely related to type b strains and which may be capsule-deficient mutants of type b strains have occasionally been observed (5, 9), this strain does not appear to be a capsule-deficient mutant of a type b strain. Thus, the occurrence of this infection 6 weeks after immunization with *H. influenzae* type b vaccine is likely to be coincidental, and this case is not a failure of the *H. influenzae* type b vaccine. The concentration in serum of anticapsular antibody at the time of his illness, although representing a suboptimal response to immunization, was likely to be sufficient for protection against type b disease (4).

We were unable to identify a host defense defect which would predispose this child to bacteremia (15). The clinical presentation of this child was of occult bacteremia with an associated febrile seizure. Occult or unsuspected bacteremia is usually due to *Streptococcus pneumoniae* or *H. influenzae* type b (11). Although cases of systemic infection due to nontypeable *H. influenzae* are now increasingly recognized in neonates (13) and may occasionally occur in normal or immunocompromised children (2, 7, 8, 10), to our knowledge, this is the first case of primary bacteremia (i.e., bacteremia without a definable tissue focus) due to nontypeable *H. influenzae* in a child. This case underscores the importance of careful serotyping of disease isolates of *H. influenzae*.

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