Non-Type b Haemophilus influenzae Infections in Adults with Reference to Biotype

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We report three cases of serious non-group b Haemophilus influenzae infections. The significance of these isolates with respect to both biotypes and serotypes is discussed.

Haemophilus influenzae are small, pleomorphic gram-negative organisms which may be classified both serologically and biochemically. Among the six serotypes, 95% of acute infections are caused by serotype b, and the sites of infection are typically the meninges, pharynx, sinus, middle ear, and lungs (19). The increased virulence of type b H. influenzae has been attributed to the carbohydrate structure of its capsule (11). More recently, however, the importance of an additional virulence factor related to biotypes 1 and 2 has been postulated (1, 9). We recently encountered three cases of non-type b H. influenzae infections in adults and were interested to note the unusual sites of infection and that they belonged to biotypes 1 or 2.

CASE REPORTS

Case 1. Case 1 was a 55-year-old black woman admitted because of fever, abdominal tenderness, and confusion. She was well until 2 weeks before admission, when she noticed shoulder pain and swelling, fever, and finally confusion. Past history was positive only for pulmonary tuberculosis 15 years ago, which was treated for 9 months with two drugs. Before the present illness, she had a chronic cough, but denied weight loss or night sweats.

On examination she had a rectal temperature of 38.2°C, blood pressure of 110/70 mm Hg, heart rate of 90/min, and respiratory rate of 18/min. She was thin, uncooperative, and disoriented. There was warmth, swelling, and erythema over the soft tissue of the right shoulder, right upper back, and left lower abdomen. These areas were tender and fluctuant. Examination of chest and heart revealed no abnormalities. There was no nuchal rigidity. A chest X ray showed an old calcified granuloma and fibrosis in the left upper lobe. The leucocyte count was 10,000/mm³, with 84% neutrophils, 4% band forms, and 12% lymphocytes. A barium enema performed before incision and drainage did not indicate contiguity between the large bowel and the abdominal abscess. During surgery, all abscesses were noted to be subcutaneous and yielded green-tinged pus which grew H. influenzae. She was begun on ampicillin, 12 g/day intravenously, after surgery.

She became afebrile after surgery and recovered uneventfully. Culture of pus obtained from abscesses on her abdomen and her shoulder grew pure cultures of H. influenzae. All isolates were mucoid, serotype a, and biotype 1. A blood culture also grew H. influenzae with the same serotype and biotype (Table 1).

Case 2. Case 2 was a 69-year-old black man who was admitted because of 20-pound weight loss over 6 months and masses on his left anterior chest and on several areas of his back. He denied fever or chills. There was no previous hospitalization except for a skin graft to a burnt right arm 10 years ago. He drank alcohol regularly and had smoked a half pack of cigarettes per day for 35 years.

On examination he was afebrile and in no distress. There were multiple subcutaneous abscesses, a 15- by 10-cm mass on the left anterior chest, a 4- by 3-cm mass on the posterior cervical area, and several 2- by 2-cm nodules on the back. These were tender and warm. The chest X ray showed a density from the hilum to both the upper and inferior lobes. The leucocyte count was 25,000/mm³, with 84% neutrophils and 16% lymphocytes. Blood cultures were not taken. Pus from each of the masses grew pure cultures of H. influenzae. Biopsy of the mass on the left anterior chest showed malignant cells.

He was treated with ampicillin, and all the wounds healed except the one on the left anterior chest. All the H. influenzae isolates were mucoid, type c, and biotype 1 (Table 1).

Case 3. Case 3 was a 24-year-old black woman in good health when she delivered a normal baby 15 h after premature rupture of her membrane. She developed a fever the same day of 38.1°C. The lochia was moderate and appeared normal. Culture of her lochia showed heavy growth of H. influenzae type a, biotype 2 (Table 1). Blood cultures were taken, and ampicillin (2 g intravenously every 6 h) and gentamicin (80 mg intravenously every 8 h) were started. The next day the uterus was tender and discharging a copious, purulent lochia. Her fever persisted on day 4 after delivery. A blood culture grew the same type of H. influenzae while the patient was on antibiotics to which the organism was susceptible. The colonies of H. influenzae appeared quite mucoid.

After 5 days of antibiotic therapy, the patient’s fever abated. She was given 5 additional days of intravenous antibiotics.

MATERIALS AND METHODS

Primary isolation of organisms from clinical specimens was attempted on McConkey, blood, and choc-
ulate agars. Organisms isolated on chocolate agar and which on Gram stain were compatible with *Haemophilus* were identified as *H. influenzae* as outlined elsewhere (20). Antimicrobial susceptibility testing was performed by a method described by Thornberry et al. (16). Organisms were serotyped by using a Difco *H. influenzae* agglutination set and biotyped by the method described by Kilian (9). Organisms were considered to belong to biotype 1 if they were indole, urease, and ornithine decarboxylase positive and to biotype 2 if they were indole and urease positive but ornithine decarboxylase negative.

**DISCUSSION**

*H. influenzae* commonly colonize the human pharynx. Turk and May have shown that 80 to 90% of these isolates were non-typable (17). Of the typable strains, type a constituted 11.5%, type b constituted 60%, type c constituted 3%, type d constituted 2%, type e constituted 12%, and type f constituted 11.5%.

Type b, which causes a disproportionately large percentage of acute infections in humans, has been studied with respect to its pathogenesis in meningitis (12), capsular content (6), and its sites of predilection (8). There are, however, only sporadic case reports of non-type b *H. influenzae* infections (5, 14, 15), including meningitis (2, 3, 7, 18) and pneumonia (4, 10). During 1979 a total of 29 patients had *H. influenzae* cultured from blood or cerebrospinal fluid or both in addition to our three cases. Table 2 shows the distribution of serotypes among these isolates. It must be pointed out that our numbers are quite small; nevertheless, the distribution is not totally different from that described by Turk and May above (17). Two of our cases revealed the ability of non-type b *H. influenzae* to produce multiple pyogenic, subcutaneous infections which, to our knowledge, are the first such cases to be reported. It is interesting to postulate that the spectrum of disease caused by non-type b organisms (e.g., endocarditis, pelvic infections, and subcutaneous abscesses) may differ from that typically produced by type b *H. influenzae*.

Trials with a vaccine for type b *H. influenzae* have begun (13). Flesher and Insel have shown that antigens from capsular types a to f reacted incompletely with type b antiserum (6). Whether any significant immunity will be engendered with the type b *H. influenzae* vaccine to other serotypes remains speculative. Such immunization may act as a selective pressure, producing a shift in the incidence of various serotypes causing disease. If the spectrum of disease for non-serotype b *H. influenzae* differs from that of serotype b, a re-evaluation of which specimens require primary isolation on chocolate agar may be required.

If the importance of the capsular virulence factor wanes with immunization, the significance of biotype as a marker for virulence may become more apparent. It appears that certain biotypes, regardless of the serotype, may have increased invasive potential. Kilian showed that *H. influenzae* could be consistently grouped according to their indole, urease, and ornithine decarboxylase reactions into five and possibly six biotypes. Moreover, the percentage of encapsulated organisms differs with the biotype, with only biotypes 1, 2, and 4 having any encapsulated organisms in his series (11). At our institution we have begun biotyping isolates that were either ampicillin resistant or from patients with meningitis. During 1979 we had three patients with ampicillin-resistant organisms and an additional seven patients with cerebrospinal fluid isolates which were ampicillin sensitive. All three ampicillin-resistant isolates were biotype 1, whereas six of seven cerebrospinal fluid isolates were biotype 1 and the other was a biotype 2. With the addition of our three patients with

### Table 1. Biochemical reactions of our isolates of *H. influenzae*, their biotype, and source

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Required hemin</th>
<th>Required NAD*</th>
<th>Indole</th>
<th>Urease</th>
<th>Ornithine decarboxylase</th>
<th>Biotype</th>
<th>Source</th>
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<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
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<td>+</td>
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<td>Yes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1</td>
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<tr>
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<td>Yes</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>2</td>
<td>Blood and lochia</td>
</tr>
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</table>

*a NAD, Nicotinamide adenine dinucleotide.

### Table 2. Distribution of *H. influenzae* by serotype at Kings County and Downstate Medical Centers for 1979 to 1980

<table>
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<th>Source</th>
<th>Serotype distribution (%)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>b</td>
</tr>
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</tr>
<tr>
<td>All ages</td>
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<td>Adults†</td>
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<td>CSF isolates</td>
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<tr>
<td>Adults</td>
<td>None</td>
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* Represents isolates from blood, cerebrospinal fluid (CSF), and patients presented in our case reports.
† Youngest person in this group was 24 years of age.
non-type b *H. influenzae*, we then have biotyped 13 isolates, 11 of which were biotype 1 and 2 of which were biotype 2. Albritton et al. determined that 42 of 43 antibiotic-resistant *H. influenzae* belonged to biotype 1 or 2 (1). Correlating biotype to the site of isolation, Albritton et al. further speculated on the potential of biotype 2 organisms in establishing genital infections. This is, perhaps, reinforced by the isolation of a biotype 2 organism in our patient with endometritis.

Many laboratories do not routinely plate wound specimens on chocolate agar and, hence, if *H. influenzae* were present, they would not be isolated. Furthermore, many laboratories do not type their *H. influenzae* isolates and only a few biotype them. We feel that these procedures would help delineate the epidemiology of non-serotype b *H. influenzae* as well as the significance of biotype markers in virulence and antibiotic resistance.

LITERATURE CITED