Antibody Responses to Capsular Polysaccharide, Lipopolysaccharide, and Outer Membrane in Adults Infected with *Haemophilus influenzae* Type b

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The antibody response to *Haemophilus influenzae* type b antigens as capsular polysaccharide, lipopolysaccharide, and outer membrane components was studied by enzyme-linked immunosorbent assay in 10 adults infected with this bacterium. Almost all patients had detectable amounts of immunoglobulins G and M antibodies specific to capsular polysaccharide, lipopolysaccharide, or outer membrane in their first serum sample. A significant antibody response in one or more antibody subclasses to capsular polysaccharide, lipopolysaccharide, and outer membrane was noted in 9 of 10, 7 of 10, and 7 of 10 patients, respectively. The occurrence of *H. influenzae* type b infections in adults cannot be completely explained either by the absence of antibodies against *H. influenzae* type b in their serum or by the failure to develop specific antibodies to the capsule or certain cell wall components even if these factors probably are important in some cases, e.g., in one patient who was splenectomized.

*Haemophilus influenzae* type b infections occurs almost exclusively in children and are rarely seen in adults (7, 13). However, during the last decade, several studies have drawn attention to *H. influenzae* infections in adults (6, 8, 10, 12, 14, 15). It is not possible to determine from the literature whether there has been a true increase in *H. influenzae* infections in adults or whether the large number of papers reflects an increased awareness of the condition. The rare occurrence of these infections in adults has been associated with the presence of protective antibodies against capsular polysaccharide (CPS) type b in adult sera (7, 11) as a result of either previous exposure and immunological response to these bacteria or exposure to other bacteria with antigens cross-reacting with polysaccharide type b (11). Even though it is known that most adults with *H. influenzae* infections have conditions predisposing them to infections, little is known about the immunology of infections caused by capsulated *H. influenzae*.

The aim of the present study was to investigate the preexisting antibody levels and the antibody response to different capsular polysaccharide, lipopolysaccharide (LPS), and outer membrane (OM) antigens of *H. influenzae* in adults infected with this bacterium.

**MATERIALS AND METHODS**

**Patients.** Serum samples from 10 patients with *H. influenzae* type b infections were analyzed. Clinical characteristics of the patients are summarized in Table 1.

**Antigens.** CPS was prepared from *H. influenzae* type b (strain RAB) as previously described (2). The concentrated culture supernatant was precipitated with cetavlon and gel filtered through Sephadex 2-B. LPS from a non-encapsulated mutant of *H. influenzae* type b (strain RAB) was prepared by extraction with hot phenol water (1). An OM preparation of the aforementioned non-encapsulated mutant was made as previously described (3).

**Antibody determination.** Enzyme-linked immunosorbent assay was performed on microplates coated with CPS, LPS, and OM antigens in concentrations of 30, 50 to 75, and 100 μg/ml, respectively (1, 2). The serum samples were tested in serial 10-fold dilutions, and antibodies were demonstrated with alkaline phosphatase-coupled swine antibodies against human immunoglobulin to immunoglobulin G (IgG), IgM, and IgA (Orion, Helsinki, Finland); the antibody concentration in the serum was expressed as $-\log_{10}$ value of the highest serum dilution showing an extinction value of 0.2 above the background. The titer assigned to each sample was the mean of two determinations. A threefold (0.5 $-\log_{10}$) or greater increase in titer was defined as significant.

**RESULTS**

The antibody responses to CPS type b, LPS, and OM of the 10 adults studied are shown in Fig. 1, 2, and 3, respectively. All patients had detectable amounts of IgG and IgM antibodies specific to CPS of *H. influenzae* type b (Fig. 1) in their first serum samples, which were obtained within 1 to 10 days after onset of symptoms. The IgG and IgM titers in the first serum samples ranged from 1.4 to 2.6 and from 1.2 to 2.3 $-\log_{10}$, respectively. IgA antibodies were detected in 6 of 10 initial serum samples.

A significant antibody response in one or more subclasses was registered in all patients except one. Significant increases in IgA, IgM, and IgG antibodies were seen in six, nine, and eight patients, respectively. The splenectomized patient (no. 3) failed to develop an increase in any of the three antibody subclasses. Two patients, aged 38 to 43 years, with no known immunodeficiencies (nos. 5 and 6), developed no IgG antibodies but had pronounced increases in IgM antibodies. The other seven patients, including no. 2 with hypogammaglobulinemia, responded with both IgM and IgG antibodies.

The kinetics of the antibody response to CPS and the maximum levels achieved were highly variable (Fig. 1). In the two patients with initial antibody response, in whom it was possible to follow antibody levels for more than 3 months (nos. 2 and 9), the IgG and IgM levels remained high during the whole observation period of ca. 5 months.

The antibody responses to LPS are shown in Fig. 2. All patients except one (no. 2) had detectable amounts of both
TABLE 1. Clinical characteristics of 10 patients with *H. influenzae* infections

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Predisposing condition</th>
<th>Infectious manifestation</th>
<th>Source of isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>F</td>
<td>Pregnant, week 35</td>
<td>Meningitis, sinusitis</td>
<td>Bl, Csf</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>F</td>
<td>Hypogammaglobulinemia</td>
<td>Epiglottitis</td>
<td>Bl, Nph</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>F</td>
<td>Splenectomized</td>
<td>Pneumonia, septic shock</td>
<td>Bl, Nph</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>F</td>
<td></td>
<td>Epiglottitis</td>
<td>Bl</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>M</td>
<td>(Mitral stenosis)</td>
<td>Epiglottitis</td>
<td>Bl</td>
</tr>
<tr>
<td>6</td>
<td>43</td>
<td>F</td>
<td></td>
<td>Pneumonia</td>
<td>Bl</td>
</tr>
<tr>
<td>7</td>
<td>43</td>
<td>F</td>
<td></td>
<td>Meningitis</td>
<td>Csf</td>
</tr>
<tr>
<td>8</td>
<td>46</td>
<td>M</td>
<td></td>
<td>Sinusitis, septicaemia</td>
<td>Bl</td>
</tr>
<tr>
<td>9</td>
<td>57</td>
<td>F</td>
<td>Chronic thyroiditis</td>
<td>Pleuropneumonia, pericarditis</td>
<td>Bl, Pl</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>F</td>
<td></td>
<td>Epiglottitis</td>
<td>Nph</td>
</tr>
</tbody>
</table>

* Bl, Blood; Csf, cerebrospinal fluid; Pl, pleural fluid; Nph, nasopharyngeal smear.

FIG. 1. Antibody response to type b CPS in 10 patients with *H. influenzae* type b infection (patient numbers correspond to those in Table 1). Day 0 = onset of symptoms.
Antibody response to type b LPS in 10 patients with *H. influenzae* type b infection. Day 0 = onset of symptoms. See Fig. 1 for definition of symbols.

IgG and IgM antibodies in their first serum sample. The IgG and IgM antibody titers ranged from 1.4 to 3.75 and from 1.0 to 3.3 −log_{10}, respectively. IgA antibodies were detected in 8 of 10 serum samples.

A significant antibody increase in one or more subclasses was noted in 7 of 10 patients. The three patients who failed to develop an antibody increase to LPS were no. 3, 7, and 8. Two of these patients (no. 7 and 8) had high antibody titers already in their first serum sample; the third patient was splenectomized.

The antibody response to OM is shown in Fig. 3. In the first serum sample, antibodies of IgG, IgM, and IgA subclasses were detectable in all 10 patients. The IgG and IgM titers ranged from 2.2 to 3.5 and from 1.1 to 3.2 log_{10}, respectively. A significant antibody response in one or more subclasses was noted in 7 of 10 patients. The patients who did not respond were no. 3, 5, and 8.

The patterns of the antibody response to OM and, especially, to LPS were highly variable (Fig. 2 and 3).

DISCUSSION

This study examined the antibody response to CPS, LPS, and OM in adults with invasive *H. influenzae* type b infections. All but one of the 10 patients studied had, in their first serum sample, levels of specific IgG and IgM antibodies to CPS comparable to levels seen in healthy adults (1). Even though the serum antibody levels before the start of infection are not known, the time between onset of symptoms and the first serum sample was in most cases so short that it may be assumed that the patients developed their infection despite preexisting specific IgM and IgG antibodies. The only patient with low initial antibodies was a splenectomized woman with very low IgM antibodies but with IgG antibodies of 2.1 −log_{10} on day 5 of infection, which is close to the mean level in healthy adults (1). This splenectomized woman was also the only patient who failed to develop a significant antibody increase to CPS in any of the three antibody subclasses. It is well known from other studies that splenec-
tomized patients sometimes respond poorly to polysaccharides of another encapsulated organism, *Streptococcus pneumoniae* (16), and that they are highly susceptible to infections with this organism (14). Splenectomized patients are also at risk of becoming infected with *H. influenzae* (4), and the absence of an antibody response in our splenectomized patient suggests that this susceptibility might be due to an inability to develop protective antibodies after exposure.

Two middle-aged patients with no known immunodeficiencies failed to develop a significant increase in IgG antibodies against type b polysaccharide, but they both had pronounced increases in IgM and IgA antibodies. Their age makes it unlikely that they should not previously have been exposed to *H. influenzae* type b. Since they failed to develop IgG antibodies, which are responsible for lasting immunity, one explanation for the development of *H. influenzae* type b infection in some adults might be an inability of certain individuals to develop protective IgG antibodies after infection. This explanation, however, cannot be valid for all adults with *H. influenzae* type b infections, since six of the patients studied here developed their invasive infections despite normal levels of specific IgG and IgM antibodies and all of these adults were capable of responding to the infection with further antibody increases. In such patients, other explanations for the development of invasive *H. influenzae* type b infections must be considered.

Antibodies to other components of *H. influenzae* are probably considerably less important for protective immunity than are antibodies to CPS. It has been shown in children that antibodies to LPS do not confer protection (9). It has been suggested, however, that antibodies to components other than CPS might contribute to the immunity, since naturally acquired antibodies to CPS offer protection at lower serum levels than antibodies induced by a purified CPS vaccine (5). The results of the present study indicate that invasive infections in adults with *H. influenzae* type b can occur in the presence of serum antibodies against both LPS and OM. The majority of our adult patients infected with this organism were also capable of producing serum antibodies of different subclasses to both of these bacterial components as well as to CPS.

The occurrence of invasive *H. influenzae* type b infections in adults therefore cannot be completely explained by either the absence of previous exposure to the organism or by a failure of the individuals affected to develop specific antibod-
ies to the capsule or certain cell wall structures, even though these factors probably are of importance in some cases.

LITERATURE CITED