Two Cases of *Mycobacterium haemophilum* Infection in Canada

LOUISE THIBERT,1* FRANÇOIS LEBEL,2 AND BERNARD MARTINEAU3

*Mycobacteriology, Québec Public Health Laboratory, Ste-Anne-de-Bellevue, Quebec H9X 3R5;1 Department of Microbiology, Montreal General Hospital, Montreal, Quebec H3G 1A4;2 and Department of Microbiology, Hôpital Ste-Justine, Montreal, Quebec H3T 1C5.3 Canada

Received 5 July 1989/Accepted 4 December 1989

In 1987, *Mycobacterium haemophilum* was isolated from cutaneous lesions, a lymph node, and the right eye of a male patient with acquired immunodeficiency syndrome and also from a cervical lymph node in a 3-year-old girl. These two cases are the first *M. haemophilum* infections to be reported in Canada.

*Mycobacterium haemophilum* was first described and named by Sompolinsky et al. (16). It is an acid-fast bacillus that requires hemin or an other iron source to grow. Of the 16 reported cases from Israel, Australia, the United States, and France, all but four occurred in patients who had lymphoma or were renal transplant recipients with infections of the skin and subcutaneous tissues (1, 4, 6, 8, 11, 13, 15, 16). This organism was once cultured from the submandibular lymph node of an infant (5) and, more recently, from cutaneous and subcutaneous lesions in three acquired immunodeficiency syndrome patients (10, 14). In this paper, we report the first two documented Canadian cases: one patient with a clinical diagnosis of acquired immunodeficiency syndrome and an otherwise healthy child with lymphadenitis.

**Patient 1.** In November 1986, at the Montreal General Hospital, a 55-year-old homosexual male who had acquired immunodeficiency syndrome (Kaposi's sarcoma and cryptococcal meningitis) and had recently resided in Rio de Janeiro presented with a pretribial soft tissue swelling and ulcer of the left leg from which *M. haemophilum* was isolated after repeated negative routine cultures. The Québec Public Health Laboratory received a BACTEC 12B vial from which growth had been detected at 30°C and confirmed by a Ziehl-Neelsen stain but from which subcultures on Lowenstein-Jensen medium (LJ) were negative. *M. haemophilum* was suspected. LJ, LJ plus 2.5% ferric ammonium citrate (FAC), Middlebrook 7H10 agar, Middlebrook 7H9 broth, and chocolate agar were inoculated and incubated at 32 and 36°C. Growth was optimal at 32°C on the media containing hemin and FAC after 2 weeks. No growth was visible on LJ. Growth in 7H9 at both temperatures and light growth on 7H10 at 32°C was attributed to the very small amount of FAC normally included in these media. On chocolate agar the colonies were nonchromogenic, rough in appearance, opaque, and flat, with a raised center and an irregular edge. On LJ plus FAC, they had a very smooth aspect. The biochemical reactions of the isolate were characteristically negative for the tests performed by standard methods, except for a positive pyrazinamidase test (9). On subsequent subcultures, growth was obtained on 7H10 with the X-factor strip at 32 and 36°C and also, to a lesser extent, on blood agar (heart infusion and tryptic soy [Difco Laboratories] bases). The isolate was sent to the Centers for Disease Control for confirmation by high-performance liquid chromatography analysis of the mycolic acids (2; G. P. Kubica and J. O. Kilburn, personal communication). Over a period of 9 months, four more isolates of *M. haemophilum* were recovered from abscesses on the right hand and leg as well as from the vitreous of the right eye and a lymph node collected at autopsy in August 1987.

**Patient 2.** In June 1987, a 3-year-old girl was referred to Hôpital Ste-Justine in Montreal because of a small submandibular lump noticed 2 months earlier by her parents. The child seemed otherwise to be in good health. Previously, in December 1986, she had been hospitalized for repairs of numerous superficial cavities. The dental work was done under general anesthesia, and the gingivae were in good condition. She was hospitalized on 18 June, and the Mantoux tests were read as follows: purified protein derivative (PPD)-S (*M. tuberculosis*), 17 mm; PPD-B (*M. intracellulare*), 15 mm; PPD-G (*M. scrofulaceum*), 15 mm; and PPD-Y (*M. kansasii*), 15 mm. On 22 June, the submandibular lymph node was biopsied. No acid-fast bacilli were found, but the pathologist concluded that the histological findings were consistent with mycobacterial lymphadenitis, and the excision of all involved nodes was recommended and done on 30 June. The histological examination of the excised nodes showed a very small number of acid-fast bacilli. On both occasions, portions of the submandibular lymph node were cultured on LJ and brain heart blood agar at 28 and 37°C for isolation of mycobacteria and fungi. Cultures for mycobacteria on LJ were all negative, but those on brain heart blood agar grew a few nonpigmented colonies after 6 weeks of incubation at 28°C. A subculture on LJ was sent to the Québec Public Health Laboratory as an inoculated LJ slope but with no apparent growth. An acid-fast stain on scrapings of the slope confirmed the presence of mycobacteria. Since it was not known that the original isolate had been obtained only on a blood-containing medium at 28°C, LJ plus 2.5% FAC, 7H10 plus X-factor, and chocolate agar were only inoculated for incubation at 32 and 36°C several weeks later, after unsuccessful subcultures on LJ and 7H10. The media at 32°C yielded fully mature nonpigmented colonies after 2 weeks. The isolate was identified as *M. haemophilum* on the basis of the growth requirements and the biochemical reactions (9). Confirmation by high-performance liquid chromatography analysis was obtained from the Centers for Disease Control (2; G. P. Kubica and J. O. Kilburn, personal communication).

Susceptibility testing. MICs of various antimicrobial agents were determined by a microdilution method in Middlebrook 7H9 broth (18) with and without hemin. When added, hemin was used at a concentration of 39 mg/liter (16). A susceptible strain, *M. tuberculosis* H37Rv (TMC 102), was used as a control. The agents tested included isoniazid, rifampin,
TABLE 1. MICs of antimicrobial agents in the microdilution broth system

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Patient 1 (32°C)</th>
<th>Patient 2 (32°C)</th>
<th>M. tuberculosis (36°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No hemin</td>
<td>Hemin</td>
<td>No hemin</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Rifampin</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>32</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>64</td>
<td>64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>p-Aminosalicylic acid</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>16</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Amikacin</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>4</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

ethambutol, streptomycin, p-aminosalicylic acid, capreomycin, gentamicin, amikacin, kanamycin, cefoxitin, and sulfamethoxazole. The plates were incubated without CO2 at 32°C for M. haemophilum and at 36°C for M. tuberculosis and read after 2 weeks. With or without hemin, both of our isolates were found susceptible to rifampin, kanamycin, amikacin, and cefoxitin but resistant to isoniazid, ethambutol, p-aminosalicylic acid, gentamicin, and sulfamethoxazole (Table 1). There was a good correlation between the MICs obtained with and without hemin. The only major discrepancy was encountered with isoniazid and the M. tuberculosis control strain, for which the MIC was much higher with the addition of hemin, an observation previously reported by Fisher (7).

The incidence of M. haemophilum infections is unknown. Its unusual growth requirements explain, at least partly, the fact that the organism is rarely isolated from patients. These two Canadian cases represent the fourth documented case involving an acquired immunodeficiency syndrome patient and the second case associated with cervical lymphadenitis in a young child. Sixteen of the 18 cases documented so far have been associated with immunocompromised patients. M. haemophilum has to be considered an opportunistic pathogen for such patients. Primary cultures on media such as LJ plus FAC, Middlebrook 7H10 with hemolyzed sheep erythrocytes or hemin, blood agar, and chocolate agar incubated at 30 to 32°C for 6 to 8 weeks should be included in cases of suspicious cutaneous and subcutaneous lesions (14) and added for lymph node biopsies when a direct smear shows acid-fast bacilli but the culture is negative. If a blood-enriched medium is already included in the battery of inoculated media, e.g., for the isolation of fungi at 30°C, it should be incubated for at least 6 weeks and examined for the presence of mycobacteria. Middlebrook 7H10 plus an X-factor supplement has been proposed as a readily available medium (17) but has never been tested for primary isolation. Although two isolates from our first case were detected in BACTEC 12B vials without additional iron, the recovery of M. haemophilum in the radiometric system benefits from the addition of an iron supplement (3).

Next to M. tuberculosis, the M. avium complex is often associated with cervical lymphadenitis in children (19). To our knowledge, for our second case as well as for the only other reported case (5), the M. haemophilum isolate was the etiologic agent and the child had no evidence of immunological deficiency. It was reported that the trauma caused to the gingivae by a needle for the administration of a dental anesthetic agent was most probably the portal of entry for a cervical lymph node M. chelonae infection (12). In our case, there was no specific event that we could relate to the presence of mycobacteria in the lymph node except for the trauma presumably associated with the repair of cavities. In view of these two cases, M. haemophilum should be suspected in children with superficial lymphadenitis, especially with a positive smear and a negative culture.

Our susceptibility testing results and those obtained by others (1, 6, 8, 10, 11, 13–16) suggest that M. haemophilum is an organism susceptible to rifampin but resistant to isoniazid, ethambutol, p-aminosalicylic acid, streptomycin, and sulfamethoxazole. Other drugs have only been tested with two or three strains, too small a number to draw any conclusions.

LITERATURE CITED


