High Rate of Tuberculosis Reinfection during a Nosocomial Outbreak of Multidrug-Resistant Tuberculosis Caused by *Mycobacterium bovis* Strain B

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We present a study of a nosocomial outbreak of multidrug-resistant tuberculosis caused by *Mycobacterium bovis* in 31 patients, 30 of whom were infected with human immunodeficiency virus; all 31 died of progressive tuberculosis. All *M. bovis* strains had identical spoligotyping patterns and showed resistance to 12 antituberculosis drugs. Reinfection was suggested in 11 cases and confirmed in 4 by molecular typing methods. The causative strain was named “B strain.”

An outbreak of multidrug-resistant (MDR) tuberculosis (TB) among patients infected with HIV caused by a *Mycobacterium bovis* strain with an unusual pattern of multidrug resistance involving 2 hospitals has been described elsewhere [1, 2]. We now describe in detail the clinical and epidemiological data from the nosocomial outbreak from 1 of these hospitals that involved 31 patients who were diagnosed from January 1995 through December 1998. The molecular epidemiology of 20 strains from this outbreak has been reported elsewhere [1].

All the mycobacteria isolates were identified as strains of *Mycobacterium tuberculosis* complex. These strains were typed by restriction fragment length polymorphism or by spoligotyping, as described elsewhere [1]. The DNA typing analysis indicated that a single MDR *M. bovis* strain caused the outbreak, and this strain has been named the MDR *M. bovis* B strain. All the strains had 2 IS6110 bands at the same positions and an identical spoligotyping pattern. In all 31 cases, the strains of *M. bovis* showed resistance to isoniazid, rifampicin, ethambutol, pyrazinamide, streptomycin, aminosalicylic acid, clarithromycin, ethionamide, ofloxacin, capreomycin, cycloserine, and amikacin. All the *M. bovis* isolates had the same microbiological characteristics; they were slow growing (mean growth time in Lowenstein-Jensen medium, 57 days), niacin negative, and nitrate negative.

Sequence analysis of 1 of the strains revealed a cytosine residue found at position 169, characteristic of *M. bovis*, which confers pyrazinamide resistance [3]. A Met 306 Ile mutation in the embB gene, a Lys 43 Arg mutation in the rpsL gene, and a Ser 531 Leu in the rpoB gene were also identified, which could account for the resistance to ethambutol, streptomycin, and rifampicin, respectively. The study of the molecular basis for isoniazid of this MDR *M. bovis* strain is ongoing.

Of the 31 cases, 30 were patients infected with HIV, 27 of whom were men and 3 of whom were women (mean age, 35 years). The HIV risk factors were as follows: injection drug users (*n* = 17), homosexual contact (*n* = 8), heterosexual contact (*n* = 4), and unknown (*n* = 1). A total of 29 patients met criteria for AIDS before they were diagnosed with the MDR B strain. One patient was a 60-year-old man without HIV infection (patient 30) whose only immunocompromising condition was alcoholism. The median CD4+ lymphocyte count at the time MDR B strain infection was diagnosed was 17 cells/μL (range, 1–162 cells/μL). All patients had multiple positive cultures and progressive TB. All of the patients died. The mean (±SD) survival time was 49.5 ± 37 days.

Of the 31 patients, 15 had been previously diagnosed with TB before the diagnosis of MDR B strain infection was made; all were infected with HIV. In 4 patients, the clinical diagnosis of TB was made without bacteriological confirmation (patients 3, 6, 19, and 29).

In 11 patients with a bacteriologically confirmed diagnosis of TB, the median time from the previous TB to the diagnosis of MDR TB caused by B strain was 254 days (range, 74–1358 days). An antibiogram was made for 8 of 11 patients, and for 4 of 11 patients, the strain involved was spoligotyped (figure 1). For all 11 strains, there was either a change in the spoligotyping pattern or a marked difference in the biochemical and microbiological characteristics when compared with the MDR
B strain, strongly suggesting reinfection. Of the 11 bacteriologically confirmed patients with TB, all had a clinical and bacteriological response to treatment (9 received treatment with isoniazid, rifampicin, ethambutol, and pyrazinamide, and 2 received treatment with isoniazid, rifampicin, and pyrazinamide). The characteristics of the previous TB of each of these 11 patients are shown in table 1. Ten patients received anti-TB treatment during and after their exposures to the MDR B strain.

Several observational studies have demonstrated that exogenous reinfection with MDR TB can occur in patients with previous TB infection [4, 5]. In HIV-infected patients, the loss of acquired immunity to *M. tuberculosis* may facilitate reinfection by an MDR strain and probably also reinfection with drug-sensitive organisms [5]. In our study, 35% of patients presented bacteriologically proven TB before the MDR B strain infection was diagnosed.

The results of this study cannot be extrapolated to determine the frequency of reinfection that might occur in other HIV-infected patients with TB, but they do suggest that in areas with high levels of HIV infection and TB, the risk of reinfection is high. Ten of the patients received anti-TB therapy during and after their exposure to the MDR B strain, and this did not prevent the development of MDR B strain infection in all 10. This indicates that even patients on standard anti-TB medication are at risk of superinfection by MDR strains of the *M. tuberculosis* complex [6].

These findings also have 2 important implications for the chemoprophylaxis of TB. First, in people with advanced HIV infection and a previous history of TB who are exposed to an infectious patient, chemoprophylaxis with an appropriate drug must be started, even in those patients who have completed a course of treatment. Second, patients who receive chemoprophylaxis or treatment for TB and who are exposed to TB caused by a strain resistant to the drugs administered may be potentially susceptible to reinfection, and an appropriate chemoprophylaxis based on drug susceptibilities must be started; otherwise, reinfection with a resistant strain could occur.

The Málaga University Hospital, Málaga, Spain, is a 710-bed hospital that cares for a population of 407,000. Until November 1995, the infectious diseases unit consisted of 21 beds in 8 double-bed and 5 single-bed rooms with a ventilation system that provided the rooms with nonrecirculated air at slight positive pressure with 6 air changes per hour (unit A). Respiratory isolation was used only for patients with bacteriologically confirmed TB.

From November 1995 through March 1996, a series of in-

### Table 1. Characteristics of the previously diagnosed tuberculosis-susceptible strain in patients with nosocomial multidrug-resistant (MDR) tuberculosis caused by *Mycobacterium bovis* B strain.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time to MDR B strain, d</th>
<th>Time of growth, d</th>
<th>Spoligotyping</th>
<th>Drug sensitivity test</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>277</td>
<td>12</td>
<td>Not available</td>
<td>Sensitivity to INH, Rif, Eth, Stm, Asa, Ethi</td>
</tr>
<tr>
<td>10</td>
<td>169</td>
<td>13</td>
<td>Not available</td>
<td>Sensitivity to INH, Rif, Eth, Stm, Asa, Ethi</td>
</tr>
<tr>
<td>11</td>
<td>254</td>
<td>20</td>
<td>Not available</td>
<td>Sensitivity to Rif, Eth, Stm, Asa, Ethi; resistance to INH</td>
</tr>
<tr>
<td>12</td>
<td>106</td>
<td>13</td>
<td>Available^c^</td>
<td>Sensitivity to Rif, Eth, Stm, Asa, Ethi; resistance to INH</td>
</tr>
<tr>
<td>14</td>
<td>288</td>
<td>18</td>
<td>Not available</td>
<td>Sensitivity to INH, Rif, Eth, Stm, Asa, Ethi</td>
</tr>
<tr>
<td>15</td>
<td>314</td>
<td>18</td>
<td>Not available</td>
<td>Sensitivity to INH, Rif, Eth, Asa, Ethi; resistance to Stm</td>
</tr>
<tr>
<td>16</td>
<td>98</td>
<td>15</td>
<td>Available^c^</td>
<td>Sensitivity to INH, Rif, Eth, Stm, Asa, Ethi</td>
</tr>
<tr>
<td>20</td>
<td>255</td>
<td>32</td>
<td>Not available</td>
<td>Not available</td>
</tr>
<tr>
<td>21</td>
<td>131</td>
<td>16</td>
<td>Available^c^</td>
<td>Sensitivity to Rif, Eth, Stm, Asa, Ethi; resistance to INH</td>
</tr>
<tr>
<td>25</td>
<td>74</td>
<td>16</td>
<td>Available^c^</td>
<td>Sensitivity to Rif, Eth, Stm, Asa, Ethi; resistance to INH</td>
</tr>
<tr>
<td>28</td>
<td>1358</td>
<td>28</td>
<td>Not available</td>
<td>Not available</td>
</tr>
</tbody>
</table>

**NOTE.** Niacine test was positive for all patients. Asa, aminosalicylic acid; B strain, MDR strain of *M. bovis* that caused the nosocomial outbreak; Eth, ethambutol; Ethi, ethionamide; INH, isoniazid; Rif, rifampicin; Stm, streptomycin.

^a^ Days from previous tuberculosis to diagnosis of MDR B strain.

^b^ Days from inoculation onto Lowenstein-Jensen medium to growth of the previous tuberculosis strain.

^c^ Figure 1 shows the spoligotyping of the previous tuberculosis strain and the MDR B strain.
Infection control measures were introduced that included the following: (1) Every patient infected with HIV who experienced respiratory symptoms or pyrexia of unknown origin was suspected to have TB. (2) We isolated patients with suspected TB in unit A. (3) All rooms in unit A had automatic door closure. (4) All visitors and health care workers entering the room had to use a high-efficiency particulate air (HEPA) mask. (5) A respiratory isolation unit (unit B) was opened comprising 10 single-bed rooms under negative pressure with the air evacuated through HEPA filters. (6) Patients with bacteriologically documented TB were transferred to unit B and isolated. (7) All patients possibly exposed to the MDR B strain were considered infected and screened for TB, regardless of the reason for their admission to unit A. In these cases, the isolation procedures were continued until the patients were either discharged from the hospital or another condition was diagnosed that subsequently responded to treatment.

After the introduction of these infection control measures, no new MDR B strain nosocomial infections were detected, underlining the importance of prevention in the control of MDR TB. In addition, from January 1998 onward, all MDR TB isolates were systematically typed in Spain (University of Zaragoza, Instituto de Salud Carlos III), providing an early warning surveillance system for outbreaks of MDR TB in Spain.

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References