Mycobacterium avium Complex: Significance of Isolation from Bone Marrow Culture

WESLEY P. KOZINN,†* BECA DAMSKER, AND EDWARD J. BOTTON

Departments of Medicine and Microbiology, The Mount Sinai Hospital, New York, New York 10029

Six patients with bone marrow cultures yielding Mycobacterium avium complex were encountered at the Mount Sinai Hospital between 1969 and 1976. One additional isolate of the same mycobacterial species was recovered from splenic cyst fluid of a seventh patient. Because none of the patients had illnesses apparently due to M. avium complex, the isolates were unexpected and unexplained. Six of the seven patients had other acute or chronic infectious processes, occurring alone or superimposed on a preexisting disease. These patients were therefore unusual, because nontuberculous mycobacteria have previously been obtained from bone marrow cultures exclusively in patients who had either disseminated or pleuropulmonary nontuberculous mycobacteriosis. The isolation of M. avium complex from the reticuloendothelial tissue of these seven patients may reflect an asymptomatic infection or alternatively may lack significance. Either premise can only be judged by continued careful evaluation of similar findings.

It is generally thought that bacillemia develops during the course of primary tuberculosis, although this occurrence is difficult to demonstrate by direct isolation of Mycobacterium tuberculosis from blood (19). Circumstantial evidence of bacillemia is the recovery of organisms from tissues which are accessible to mycobacterial seeding only by a hematogenous route (25). One such tissue is the bone marrow, which may yield Mycobacterium tuberculosis on culture in the course of the primary infection, as well as during postprimary dissemination (2, 3, 12).

There is no evidence that pulmonary disease due to nontuberculous mycobacteria is associated with hematogenous spread (1, 31). However, the isolation of causative nontuberculous mycobacteria from the bone marrow of some patients with pulmonary mycobacteriosis suggests that bacillemia may occur during the course of such infection (23), and there are rare reports of direct blood isolates of these organisms from patients with this illness (16). Bone marrow isolates have also been obtained in as many as 16 of the reported cases of disseminated nontuberculous mycobacteriosis (6–11, 13–15, 16, 22, 27, 29, 32, 33; E. H. Runyon, unpublished data). Although this is rare, asymptomatic infections with nontuberculous mycobacteria, manifested only in retrospect by skin test reactivity to mycobacterial antigens, are common (5, 30). The disease becomes apparent when pathological changes result in clinical symptoms.

Isolation of nontuberculous mycobacteria from systemic tissues of persons without clinical evidence of disease due to these species might provide documentation of an inapparent infection (24), and the location of organisms might suggest the manner of their deposition. This communication reports the recovery of Mycobacterium avium complex from the bone marrow culture of six patients and from the spleen of a seventh. These isolations were unusual and perplexing because none of these seven subjects had clinically apparent nontuberculous mycobacterial disease. The potential significance of the M. avium complex isolations from such reticuloendothelial tissues serves as the basis for this report.

MATERIALS AND METHODS

During the 7-year interval (1969–1976) of this study, 588 bone marrow specimens were processed for mycobacteria. Of these, six yielded nontuberculous mycobacteria, (M. avium complex), one yielded Nocardia asteroides, and one yielded Mycobacterium tuberculosis. The remaining bone marrow cultures were negative for acid-fast organisms. The seventh M. avium complex isolate was from splenic cyst fluid. From one of the patients an additional isolate was also recovered from the culture of a pharyngeal ulcer.

Direct smears were prepared from all specimens and stained by the Kinyoun method. Because the bone marrow specimens were presumed to be free of bacterial contaminants, a decontamination procedure was eliminated. Specimens were therefore inoculated directly onto Lowenstein-Jensen (L-J) or Middlebrook 7H10 or 7H11 slants and into a modified Dubos liquid medium (4), as well as onto blood agar plates. The throat specimen was decontaminated with filtered and autoclaved 2% sodium hydroxide, washed with saline, and inoculated as above. Cultures were incubated in 5% CO₂ at 35 to 37°C and examined at 7-day intervals.
for evidence of mycobacterial growth. Tests for niacin production, nitrate and tellurite reduction, and Tween 80 hydrolysis were performed on all isolates according to standard methods (21, 28).

Information concerning the seven patients was obtained from a review of their hospital charts, and relevant clinical, microbiological, and pathological findings are presented in Table 1. Histological sections were reviewed when available. With the exception of patient 3, bone marrow for culture was obtained through biopsy and aspiration of the posterior iliac crest after povidone-iodine and alcohol preparation and draping of the puncture site. For patient 3, marrow was obtained from sternal puncture after similar preparation. Fluid from a splenic cyst of patient 7 was obtained at the time of splenectomy.

RESULTS

Six of the seven patients in this series were hospitalized with a variety of serious infectious illnesses at the time specimens were obtained for mycobacterial culture (patients 1 to 6) (Table 1). Infectious processes were superimposed on a preexisting illness for patients 2, 3, 5, and 6. Patients 1 and 4 were thought to have *Aspergillus flavus* osteomyelitis and tuberculous pleurisy, respectively. Two of the patients (no. 2 and 3) had streptococcal endocarditis, one due to *Streptococcus bovis* and the other to an alphahemolytic species. The latter patient had a similar streptococcal species isolated from the bone marrow concomitantly with *M. avium* complex.

Patient 5 had marked hypogammaglobulinemia, anemia, and chronic debility from extensive Crohn's disease. Patient 6, who expired, had advanced Hodgkin's disease and bone marrow hypoplasia related to chemotherapy. At postmortem, disseminated fungal microabscesses were present, but histological evidence of mycobacterial disease was absent. Postmortem cultures, however, were not taken. With respect to patient 7, there were no clinical or laboratory findings to suggest that cystic replacement of the spleen had caused any defect in host immunity, and she was otherwise well.

Purified protein derivative-tuberculin tests were performed for patients 1 to 6. Induration greater than 10 mm was obtained for patient 1 with 250 tuberculin units and for patient 4 with 5 tuberculin units. Patient 3 had a nonreactive test at the time of marrow culture, but a subsequent test 2 years later showed 8-mm induration. None of the patients was skin tested with non-tuberculous mycobacterial antigens. Chest roentgenograms revealed acute inflammatory process only with patient 4. There was no evidence on the chest roentgenograms of the other patients of either acute or prior mycobacterial infection.

Mycobacteriologically, only one of the six bone marrow specimens showed acid-fast bacilli

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex; age (yr)</th>
<th>Clinical synopsis*</th>
<th>Bone marrow histology</th>
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<tbody>
<tr>
<td>1</td>
<td>F; 51</td>
<td>Heroin addict with chronic <em>Aspergillus flavus</em> osteomyelitis of lumbar 3-4; 5 TU-PPD negative, 250 TU-PPD 18-mm induration; normal chest roentgenogram</td>
<td>Normocellular; no granulomas or AFB</td>
</tr>
<tr>
<td>2</td>
<td>M; 75</td>
<td><em>Streptococcus bovis</em> SBE with meningitis responsive to penicillin therapy; 5 TU-PPD negative; normal chest roentgenogram</td>
<td>Erythroid hyperplasia; no granulomas or AFB</td>
</tr>
<tr>
<td>3</td>
<td>M; 28</td>
<td><em>Viridans</em> streptococcal SBE superimposed on rheumatic mitral stenosis, penicillin responsive; 5 TU-PPD negative, converted to 8-mm induration upon repeat testing 2 years later</td>
<td>Erythroid hyperplasia</td>
</tr>
<tr>
<td>4</td>
<td>F; 47</td>
<td>Tuberculous pleurisy resolved with INH and ethambutol; pleural biopsy—caseating granulomas with giant cells, no AFB; cultures of sputum, pleural fluid, urine, all negative for mycobacteria, 5 TU-PPD 17-mm induration</td>
<td>Normocellular; no granulomas or AFB</td>
</tr>
<tr>
<td>5</td>
<td>M; 16</td>
<td>Extensive Crohn's disease and infectious mononucleosis; normal chest roentgenogram</td>
<td>Reactive marrow with left shift; no granulomas or AFB</td>
</tr>
<tr>
<td>6</td>
<td>M; 72</td>
<td>Hodgkin's disease IVb; <em>Herpes zoster</em> of the trunk; right hilar enlargement; <em>M. avium</em> complex cultured from necrotic pharyngeal ulcer</td>
<td>Hypoplastic, consistent with chemotherapy</td>
</tr>
<tr>
<td>7</td>
<td>F; 20</td>
<td>Splenectomy; PPD not done; normal chest roentgenogram; pathology—large angiomatous cysts; no granulomas or AFB present</td>
<td>Normal</td>
</tr>
</tbody>
</table>

* TU-PPD, Tuberculin units of purified protein derivative; SBE, subacute bacterial endocarditis; AFB, acid-fast bacilli.
on direct smear (patient 6). For patients 3 and 6, initial mycobacterial isolation was obtained on both slants as well as in liquid medium, whereas for patient 5, isolation was achieved on solid media exclusively. Four bone marrow isolates (patients 1, 2, 4, and 7) and the pharyngeal isolate (patient 6) developed only in the liquid medium. None of the mycobacterial isolates developed on blood agar plates after 10 days of incubation. An alpha-hemolytic streptococcus was obtained from the marrow of patient 3.

The rate of mycobacterial growth was slow, occurring in 2 to 7 weeks of incubation at 35 to 37°C. In those instances when growth occurred on solid media (patients 3, 5, and 6), there were more than 50 nonpigmented, smooth, and domed colonies on L-J, whereas on 7H10 or 7H11 agar they were flat and transparent or opaque and domed. In the liquid medium, growth occurred either as a nonpigmented, smooth, uniform sediment or in the form of numerous fine clumps dispersed throughout the medium. Subcultures of this initial growth onto L-J and 7H11 media grew within 9 to 20 days. Colonial pigmentation was never observed despite exposure to light and prolonged incubation.

Biochemically, all eight isolates were negative for niacin production, nitrate reduction, and Tween 80 hydrolysis. Tellurite was reduced within 3 days. In the absence of serotyping which may distinguish between Mycobacterium intracellulare and M. avium, but on the basis of the observed cultural and biochemical characteristics, the isolates were identified as M. avium complex (21, 28).

DISCUSSION

Nontuberculous mycobacteria are considered to be of relatively low virulence, although there is increasing recognition of M. avium complex and others as human pathogens (1, 31). These species are acquired mainly from environmental sources such as soil, water, and dust (30). Birds and mammals may excrete them directly or in products such as eggs, milk, and their derivatives (1). Considering the frequency of skin test reactivity to PPD-Battey and other mycobacterial skin test antigens in some geographic areas, clinically occult infection with these organisms may be a common event (5). Alternatively, sensitization may develop from exposure to mycobacterial or fungal antigens in the environment and may not mean that infection has occurred (30).

In the present study, none of the seven patients had disease attributable to M. avium complex. With four patients (no. 2, 3, 5, and 6), a firm diagnosis of either bacterial, fungal, or viral infection was made. Patient 7 had no evidence of infection. The precise cause of illness remained uncertain for patients 1 and 4, both of whom had reactive tuberculin tests. Patient 4 had clinical symptomatology, pathological findings, and a response to antituberculous therapy consistent with pleurisy due to M. tuberculosis.

Because there was no evidence of nontuberculous mycobacterial disease in these seven patients, the bone marrow and splenic cyst fluid isolates of M. avium complex had no particular significance to the physicians caring for these patients. The possibility of the isolates being skin contaminants is remote, since M. avium complex is not quoted as a normal inhabitant of human cutaneous tissue (20). Contamination with M. avium complex of water used for our laboratory preparations is conceivable but improbable, since the water is both filtered and autoclaved before use. For these patients, however, it is difficult to prove a strictly endogenous source of their primary isolates. Although the recovery of M. avium complex from liquid medium does not itself exclude contamination from the skin, its sole recovery in this milieu does support an endogenous origin as do the numerous colonies developing initially on the slanted media.

The occurrence of a latent or apparent infection with M. avium complex in the seven patients described herein might provide an explanation for the reticuloendothelial tissue isolates of this organism. Assuming a primary site of infection with M. avium complex, it is possible that there was an associated bacillemia of unknown duration which was cleared by reticuloendothelial organs such as the bone marrow or spleen. The evidence of bacillemia is circumstantial, but in the absence of direct extension of infection, organisms could be deposited in the bone marrow or spleen only by a hematogenous route. Viable organisms recovered fortuitously from cultures may provide further documentation of the concept of asymptomatic infection.

The portal of entry for M. avium complex in the patients described here remains obscure. Patient 6, who was immunosuppressed, was the only individual who had M. avium complex isolated concomitantly from a site other than the bone marrow, because the organism was also recovered from a swab of a pharyngeal ulcer. One may speculate that the organism either gained access to the circulation through this ulcer or was a coincidental commensal in the oral cavity (17).

Although it remains difficult to ascribe absolute clinical significance to these M. avium complex isolates, the recovery of nontuberculous mycobacteria from cultures of bone marrow has
previously been reported almost exclusively in association with disseminated or pleuropulmonary nontuberculous mycobacterial disease (6-11, 13-15, 16, 22, 23, 27, 29, 32, 33; E. H. Runyon, unpublished data). Group III nonchromogens (i.e., “Battey” strains, M. intracellulare or M. avium) were recovered in 18 of these 29 reported instances. In addition, “unclassified atypical mycobacteria” have been isolated from the marrow of two patients with miliary tuberculosis (3). There are only two reported isolations of nontuberculous mycobacteria from the bone marrow of individuals without any evidence of mycobacteriosis, and it was proposed that such isolations represented documentation of asymptomatic infection with these organisms (24).

Alternatively, the recovery of M. avium complex from cultures of bone marrow of patients who lack evidence of disease may not at all be equated with infection (26). The above two premises, however, can only be judged by the continued careful evaluation of the recovery of nontuberculous mycobacterial species from bone marrow. In a suitable animal model, experimental demonstration of dissemination could possibly illuminate the as yet ignored pathogenesis of nontuberculous mycobacterial infection (18, 31).

LITERATURE CITED