Rapid detection and identification of mycobacteria from blood of patients with acquired immune deficiency syndrome

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Summary. A combination of radiometric broth culture (Bactec 13A) and the Gen-probe nucleic acid hybridisation system was used to detect and identify *Mycobacterium tuberculosis* and *M. avium* from the blood of patients with acquired immune deficiency syndrome (AIDS).

Introduction

With the increased incidence of the acquired immune deficiency syndrome (AIDS), the isolation of mycobacteria from blood has become more frequent,^{1–3} but methods for their isolation and identification take several weeks. However, two products have become available recently that might provide a more rapid diagnosis—a radiometric broth (Bactec 13 A; Johnston Laboratories Inc., Towson, MD, USA) specifically for the culture of mycobacteria from blood³ and a nucleic-acid hybridisation kit for the rapid identification of *M. avium*, *M. intracellulare* and the *M. tuberculosis* complex (Gen Probe Inc., San Diego, CA, USA). Identification is based on the ability of complementary nucleic acid strands to bond and form stable double-stranded complexes.^{4–6}

The purpose of this study was to investigate the use of the combination of 13A medium, recommended for the growth of mycobacteria from blood,³ and the Gen-probe system described as sensitive and specific for the identification of mycobacteria,⁶ to improve the diagnosis of mycobacterial infections in patients with AIDS and the AIDS-related complex (lymphadenopathy-associated syndrome and persistent generalised lymphadenopathy).⁷ Rapid detection and identification of *M. avium* and *M. intracellulare* is important because these micro-organisms are a major cause of disease in AIDS patients³ and because therapy for these patients differs from that used for patients infected with *M. tuberculosis*.⁶ Generally, most *M. avium-intracellulare* strains are resistant to isoniazid, rifampicin and quinolones but are susceptible to amikacin and gentamicin.

Materials and methods

Specimens

From Jan. 1988 to May 1989, 875 blood specimens for the detection of mycobacteria were obtained from 153 patients with AIDS or associated syndromes, in Civili Hospital, Brescia.

Culture methods and media

Blood (5 ml) was collected in a Bactec 13A vial (Johnston Laboratories) and incubated at 36°C. The vials were examined radiometrically daily on the Bactec 460 instrument; smears from samples with a growth index (GI) >20 were stained for acid-fast bacilli by the auramine method and examined by fluorescence microscopy. Positive samples were subcultured on Lowenstein-Jensen and International Union (against) Tuberculosis Mycobacterium (I.U.T.M.) media (Sclavo S.p.a., Via Fiorentina 1, Siena, Italy).

Identification

Vials with a GI >20 and a positive auramine film were examined with the Gen-probe rapid diagnostic system. A 5-ml sample of a positive blood culture was centrifuged (3200 rpm) for 10 min and 200 µl of the pellet was added to a tube of lysing reagent and sonicated for 15 min at 50–70°C in a water-bath sonicator. Probe solution (1 ml) was added and the tube was incubated for 1 h at 72°C in a water bath. Separation suspension (4 ml) was added and incubated at 72°C for 5 min in a water bath; the tube was then centrifuged (500 rpm) for 2 min. Wash solution was added and the suspension was centrifuged (500 rpm) for 2 min. The supernate was decanted and the tube was drained completely. Each tube was counted in a gamma counter for 1 min and hybridisation was calculated as follows: hybridisation (%) = (sample cpm – background cpm) / (total cpm of input probe – background cpm) × 100; hybridisation >10% was considered a positive

result. The growth on Lowenstein-Jensen and I.U.T.M. media was identified by classical biochemical tests that included nitrate reduction, niacin and arylsulphatase production, catalase inactivation at 68°C, photochromogenicity, tellurite reduction, Tween 80 hydrolysis and urease production.

Antibacterial susceptibility tests

In-vitro susceptibility tests for *M. tuberculosis* and *M. avium* were performed on 7H11 agar and isolates were considered susceptible when $\geq 99\%$ reduction in the number of colonies was observed on the antimicrobial plate compared with the control plate.⁸

Results

Of the 875 blood cultures taken from 153 patients with AIDS or associated diseases 82 (9.4%) cultures from 17 patients were positive for mycobacteria after an average time of 19.2 ± 7 days. The mycobacterial isolates were identified by Gen-probe—13 (15.8%) from six patients as *M. tuberculosis* complex and 69 (84.2%) from 11 patients as *M. avium*. The average growth time for *M. tuberculosis* was 27.4 SD 7 days and for *M. avium* 17.8 SD 5 days. Identification by the Gen-probe system took c. 2 h. For *M. tuberculosis*, the mean percentage hybridisation was 28.45 SEM 2.06 with the homologous probe, 1.45 SEM 0.29 with the heterologous *M. avium* probe and 1.57 SEM 0.38 with the heterologous *M. intracellulare* probe. For *M. avium* isolates, the mean percentage hybridisation with the homologous probe was 33.72 SEM 5.92, whereas with the heterologous *M. tuberculosis* complex and *M. intracellulare* probes the percentage hybridisation was 1.99 SEM 0.65 and 1.77 SEM 0.53 respectively. The percentage hybridisation with different probes for each of the 17 *Mycobacterium* strains is shown in the table. For comparison all the mycobacterial isolates grown on Lowenstein-Jensen and I.U.T.M. media were identified by classical biochemical tests: 13 isolates that failed to grow on Lowenstein-Jensen medium within 7 days, produced niacin, reduced nitrate and produced catalase that did not withstand 68°C for 20 min were identified as *M. tuberculosis*;⁹ and 69 isolates that were slow growers at temperatures from 22 to 43°C, produced smooth colonies that were not photochromogenic, became yellow on ageing, gave negative results for niacin production, nitrate reduction, Tween 80 hydrolysis, arylsulphatase activity, and urease production, and positive results for tellurite reduction and catalase activity at 68°C were identified as *M. avium*.¹⁰

Strains of *M. tuberculosis* were sensitive to etham-

butol, streptomycin, rifampicin, isoniazid, ciprofloxacin and ofloxacin. *M. avium* strains were sensitive to ethambutol, streptomycin, amikacin and gentamicin.

Discussion

Of 17 cases of mycobacterial septicaemia in 153 patients with AIDS or associated syndromes, 15.8% were caused by *M. tuberculosis* and 84.2% by *M. avium*. Two of the cases had not shown any classical symptoms of septicaemia and were diagnosed unexpectedly by detection of atypical mycobacteria from blood emphasising the value of rapid detection. Although the time necessary for bacterial growth is not reduced in comparison with other systems such as Isolator³ the use of Bactec 13A is advantageous in situations where it is desirable to avoid any further manipulations of the specimen, e.g., when taking blood at the bedside. Blood can be injected directly into the vial, ensuring greater accuracy of laboratory diagnosis and increased safety for the operators. The study also showed the reliability of the Gen-probe system, already known to be rapid and specific.^{5,6,11-13} The greatest criticism of the Gen-probe system is its high cost, but the advantage of being able to identify strains directly from a vial such as the Bactec 13A without waiting for growth of colonies on solid media is

Table. Nucleic acid probe hybridisation and classical biochemical identification of *Mycobacterium* strains

Strain no.	Percentage hybridisation with probe for		
	<i>M. avium</i>	<i>M. intracellulare</i>	<i>M. tuberculosis</i> complex
<i>M. tuberculosis</i>			
1	1.6	1.6	27
2	1.3	1.3	31
3	1.8	2.1	31
4	1.7	1.9	26.3
5	1.1	1.4	28.1
6	1.2	1.1	27.3
<i>M. avium-intracellulare</i>			
7	42	2.1	1.8
8	20.8	1.3	1.6
9	25.7	1.5	1.3
10	37.1	2.3	1.4
11	34.2	1.2	1.9
12	35.1	1.4	2.1
13	36.1	1.6	2.2
14	34.6	1.3	2.5
15	32.3	1.8	1.1
16	33.9	2.1	3.1
17	39.1	2.9	2.9

considered to justify the expense. The use of the Gen-probe system increases the usefulness of Bactec 13A, emphasising its safety and practicality. Another criticism of the Gen-probe test is that identification of *M. tuberculosis* cannot be considered final because the probe identifies only the *M. tuberculosis* complex which includes *M. africanum*, *M. bovis* and *M. microti*.¹² However, *M. bovis* is identified easily by negative results in niacin and nitrate tests; *M. africanum* is found only in tropical Africa; and *M. microti* occurs only in voles and other small mammals and may be regarded as a biovariety of *M. tuberculosis*.¹³ In our

tests, all strains identified by the Gen-probe system as *M. tuberculosis* complex were confirmed as *M. tuberculosis* by classical biochemical tests.

AIDS patients infected with *M. tuberculosis* respond satisfactorily to standard antituberculous chemotherapy. This may not be so in infections due to *M. avium* as strains are often resistant to a wide variety of antituberculous drugs. It is useful, therefore, to be able to identify the infecting organism rapidly so that adjustments to chemotherapy may be made. What constitutes correct therapy for infections caused by *M. avium* remains to be decided.

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