JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1985, p. 168–173
0095-1137/85/020168-06$02.00/0

Vol. 21, No. 2

Infections Caused by *Mycobacterium avium* Complex in Immunocompromised Patients: Diagnosis by Blood Culture and Fecal Examination, Antimicrobial Susceptibility Tests, and Morphological and Seroagglutination Characteristics

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Received 26 July 1984/Accepted 8 October 1984

The *Mycobacterium avium* complex, only rarely described as an invasive pathogen in humans, has recently been reported to frequently cause disseminated disease in patients with the acquired immune deficiency syndrome. Between February 1981 and February 1984 at Memorial Sloan-Kettering Cancer Center, 30 patients with acquired immune deficiency syndrome, 3 patients with leukemia, and 2 patients with congenital severe combined immunodeficiency syndrome developed disseminated *M. avium* complex infection. Mycobacteria were often found in multiple sites both antemortem and postmortem. Blood cultures were a reliable method for detecting disseminated infection, and the new lysis blood culture systems provided an efficient technique for determining the number of organisms per milliliter of blood. Acid-fast stains and cultures of fecal specimens were also helpful in diagnosing infection. Most of the mycobacteria were serovar 4 (77%), and most (86%) produced a deep yellow pigment. All isolates were susceptible to standard concentrations of clofazimine, cycloserine,and ansamycin, but tended to be resistant to isoniazid, streptomycin, ethambutol, ethionamide, and rifampin.

Members of the *Mycobacterium avium* complex are mycobacteria that have been isolated from several environmental sources including soil (24), water (7), and house dust (17). These organisms produce diseases in birds (14), including chickens (5), and animals, including pigs (10) and humans. In humans, the *M. avium* complex causes infections of the lungs, lymph nodes, skin, bones, soft tissues, and the genitourinary tract (2, 4). However, there were very few reports of disseminated infections with these organisms before recognition of the acquired immunodeficiency syndrome (AIDS). Recently, several reports have noted disseminated *M. avium* complex infections in AIDS patients (3, 8, 12, 16, 25; J. W. M. Gold, C. L. Weikel, M. L. Tapper, T. E. Kiehn, C. W. Lerner, C. Urmacher, H. Z. Rotterdam, and D. Armstrong, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 227, 1983).

Since 1980, at Memorial Sloan-Kettering Cancer Center, we have seen 35 cases of disseminated *M. avium* complex infection. These infections occurred in patients who were immunocompromised because of AIDS, leukemia, or severe combined immunodeficiency disease. In this paper we report cultural, morphological, and serological characteristics and in vitro susceptibility test results of *M. avium* complex isolates from these patients.

MATERIALS AND METHODS

Patients with disseminated *M. avium* complex infection had either AIDS, congenital severe combined immunodeficiency disease, or leukemia as an underlying disease. Isolates from eight patients with neoplastic disease, but who had no evidence of invasive mycobacterial disease, were also studied.

Respiratory specimens, urine, sterile body fluids, and tissues were processed by conventional procedures, including digestion and concentration of sputums with *N*-acetyl-L-cysteine and a final concentration of 1% sodium hydroxide (22). Processed specimens were then inoculated onto Middlebrook 7H11 agar and Lowenstein-Jensen medium and, when contamination with normal body flora was suspected, Mitchison selective 7H11 agar (21). Slides for detection of acid-fast bacilli were prepared by using the auramine O fluorochrome stain (18).

Acid-fast stains were made directly from unprocessed fecal material. When acid-fast organisms were detected, a suspension of feces (1 g in 5 ml of Middlebrook 7H9 broth) was processed for culture by the sputum digestion method described above.

Initially, in 1981, blood for culture was collected in a Vacutainer tube (100 by 16 mm) containing 5.95 mg of sodium polyanetholesulfonate (Becton Dickenson and Co., Paramus, N.J.). Upon arrival in the laboratory, the Vacutainer tube was centrifuged for 30 min at 1,500 × *g*. The supernatant was removed, and 0.2 ml of concentrated sediment remaining in the tube was inoculated equally onto a 7H11 agar plate and a Lowenstein-Jensen medium slant. Late in 1982 we began quantitating the level of mycobacteremia. When the clinical condition suggested high-grade bacteremia, blood was collected in the Vacutainer tube, and 10- and 100-fold dilutions of uncentrifuged blood were made with 7H9 broth as a diluent. Portions (0.1 ml) of undiluted

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and diluted blood were inoculated onto 7H11 agar plates. In 1983 we began using the Isolator-10 lysis-centrifugation system (Du Pont Co., Wilmington, Del.). The blood culture tube contains an anticoagulant and saponin, which is a lytic agent, and 10 ml of blood can be collected into this tube. After blood was collected in the Isolator-10 tube, the tube was inverted several times to ensure mixing and was delivered to the microbiology laboratory. After lysis of blood cells and concentration of the sediment in a centrifuge at 3,000 × *g* for 30 min, 1.5 ml of sediment was divided equally and inoculated onto four 7H11 agar plates. When high-grade bacteremia was expected, 10- and 100-fold dilutions of Isolator-10 sediment were also inoculated onto 7H11 agar plates. Acid-fast stains of Isolator sediment were prepared from the blood of three selected patients. Later in 1983 we also began using the Isolator-1.5 lysis tube. This tube also contains an anticoagulant and saponin; however centrifugation is not required, and only 1.5 ml of blood is collected into the tube. After the inoculated tubes arrived in the laboratory, lysate from the uncentrifuged tubes was inoculated onto agar media as desribed for Vacutainer blood. Dilutions (10- and 100-fold) of Isolator-1.5 lysate were also made when high-grade bacteremia was expected.

All cultures were incubated at 35°C in 5% CO_2 in air; Lowenstein-Jensen medium slants were incubated for 8 weeks, and 7H11 and S7H11 plates were incubated for 4 weeks. The medium was inspected for visible colonies twice each week, and the type of pigmentation was also noted. When growth of *M. avium* complex was detected on 7H11 plates from the Vacutainer dilutions or the Isolator systems, the time to detection and number of colonies per plate were recorded. The CFU per milliliter of blood was determined based on the amount of blood collected.

M. avium complex isolates, both from patients with disseminated mycobacterial infections and from patients with no evidence of invasive disease, were serotyped at National Jewish Hospital and Research Center, Denver, Colo., with seroagglutination methods originally described by Schaefer (19).

Mycobacteria were tested for susceptibility to ansamycin, clofazimine, cycloserine, ethambutol, ethionamide, isoniazid, rifampin, and streptomycin by using conventional agar dilution methods (22). The concentrations of the compounds are recorded in Table 4.

TABLE 1. Frequency of positive antemortem *M. avium* complex cultures

Specimen	No. of patients cultured	No. of positive cultures
Blood	25	24
Bone marrow	12	12
Brain	1	0
Bronchial or tracheal secretions	15	12
Cerebrospinal fluid	10	2
Intestine	6	6
Liver	6	6
Lung	1	1
Lymph node	2	2
Peritoneal fluid	2	1
Pleural fluid	3	3
Spleen	1	1
Sputum	14	9
Stool	17	12
Urine	18	9

RESULTS

From February 1981 through February 1984 at Memorial Sloan-Kettering Cancer Center, disseminated *M. avium* complex infections were diagnosed in 35 immunocompromised patients: 30 patients with AIDS, 2 young males with severe combined immunodeficiency disease, and 3 patients with leukemia. Ages ranged from 9 months to 64 years, with an average age of 35 years.

Extent of infection. *M. avium* complex was detected by acid-fast smear and isolated either antemortem or postmortem (or both) from a variety of body sites. Table 1 lists the frequency of isolation from various body sites cultured antemortem. Several specimen types, including those involving relatively noninvasive procedures such as blood, respiratory secretions, sputum, stool, and urine, were often positive for growth of the mycobacterium. *M. avium* complex was also isolated postmortem at least once from each of the following sites: adrenal gland, blood, bone marrow, brain, esophagus, eye, heart, intestine, kidney, liver, lung, lymph node, pancreas, prostate gland, spleen, stomach, testes, thymus, thyroid, and tongue.

Detection by blood culture. *M. avium* complex was recovered antemortem from the blood of 24 of 25 patients from whom blood cultures were collected; from 5 of these patients, blood was the only antemortem culture positive for mycobacteria. Colonies on 7H11 agar were usually visible by 7 to 14 days of incubation, and no additional growth of mycobacteria occurred after 21 days. Precise colony count determinations were not made on blood from five patients who were seen early in 1981. The number of organisms in the blood of six patients, where only the Vacutainer system was used, ranged from 20 to 28,000 CFU/ml of blood.

The Isolator-10 system was used routinely for blood culture from the next 13 patients, who were determined to be at high risk for *M. avium* complex infection. Dilutions of Isolator-10 sediment were made when results indicated that high colony counts (i.e., >300 CFU/ml) were expected. Nine of these patients had low-level mycobacteremia; colony counts did not exceed 59 CFU/ml of blood; thus dilutions of Isolator sediment were not needed. When three of these patients were found to have high-level mycobacteremia, we compared, at least once for each patient, the colony counts of the three quantitative blood culture systems (Table 2). Isolator-10 sediment was diluted after precise determinations from the undiluted sediment of previous cultures could not be made. The results that can be compared are the last three cultures each of patients no. 1 and 2 and the 17 February culture of patient no. 3. Colony counts for six of seven cultures were similar when the two Isolator systems, both containing the lytic agent saponin, were compared. Colony counts from the Vacutainer dilution method compared favorably to those of the lytic systems for patients no. 2 and 3, but Vacutainer colony counts were usually considerably below those of the lytic systems of patient no. 1. This patient had very high-level bacteremia that persisted until death.

Acid-fast stains of blood and feces. The stains of Isolator-10 sediment from the blood of patients 1, 2, and 3 in Table 2 were positive for acid-fast bacilli. Direct acid-fast smears were also performed on the feces from 17 patients; 12 of these smears were positive, and *M. avium* complex was isolated from all 12 cultures. The five fecal specimens that were smear negative were not cultured.

Serovars. Table 3 lists the serovars of *M. avium* complex strains from 26 patients with disseminated mycobacterial

disease and 8 *M. avium* complex isolates from cancer patients with no evidence of invasive mycobacterial disease. All isolates from disseminated cases were either serovar 1, 4, or 8; 20 of the 26 (77%) were serovar 4. These results contrast with those from patients with no invasive disease; the eight isolates either did not agglutinate in antisera, cross-agglutinated with several antisera, or were serovar 6 or 18.

Pigmentation. Of the 35 organisms from disseminated cases, 30 produced a distinct yellow pigment before exposure to light, whereas only 1 of the 8 strains from patients with no evidence of invasive disease produced this pigment. The remaining strains were the buff color usually associated with fresh clinical *M. avium* complex isolates. The five nonpigmented organisms from disseminated cases included all three serovars, 1, 4, and 8.

Susceptibility studies. Table 4 shows the results of the antimycobacterial susceptibility tests for the 35 strains of *M. avium* complex from disseminated cases. All strains were susceptible to 1μg of clofazimine per ml, 30 and 60μg of cycloserine per ml, and 2μg of ansamycin per ml. Many strains were resistant to 5μg of ethambutol and ethionamide per ml, but were susceptible to the next higher concentration; 7.5 μg of ethambutol per ml and 10 μg of ethionamide per ml. There were no consistent differences among the various serovars in antimicrobial susceptibility.

DISCUSSION

Since 1979, over 4,000 cases of AIDS have been reported to the Centers for Disease Control, and disseminated infection with *M. avium* complex has emerged as a major complication of these patients (3, 8, 12, 16, 25). At this hospital as of 1 April 1984, we have seen 226 AIDS patients; 71 have died and autopsies were performed on 55.

Disseminated *M. avium* complex infection was documented in 30 (55%) of these autopsies. Extensive dissemination was demonstrated by the numerous body sites from which the mycobacterium was often cultured, both antemortem and postmortem. The patients with and without disseminated *M. avium* complex infection had a variety of infections with organisms taking advantage of T-cell defects. These organisms included cytomegalovirus, *Pneumocystis carinii*, and *Cryptsporidium* sp.

Since this mycobacterium is now known to be a common infecting agent in certain immunocompromised patients and the disease is often fatal, new and sensitive diagnostic microbiology procedures are needed. In this study, fecal smears and culture for acid-fast bacilli and blood cultures, with new quantitative technologies, were helpful. *M. avium* complex was often detected in fecal specimens, first by acid-fast stains of feces and then by culture when stains were positive. Whether cultures of stools with negative smears should be performed on patients at high risk for this infection

TABLE 3. Frequency of *M. avium* complex serotypes from 26 patients with disseminated infection and 8 cancer patients with no evidence of invasive disease

Patients with:	Underlying disease	No. of patients	Serotype
Disseminated infection[a]	AIDS	18	4
	AIDS	3	8
	AIDS	2	1
	Severe combined immunodeficiency syndrome	2	4
	Leukemia	1	1
No invasive disease[b]	Cancer	4	No agglutination
	Cancer	2	Cross-agglutination in several antisera
	Cancer	1	6
	Cancer	1	18

[a] Isolates were from blood or deep tissues.
[b] Isolates were from respiratory or urine specimens.

TABLE 2. Quantitative blood culture methods used and colony counts for three patients with *M. avium* complex mycobacteremia

Patient no.	Date	No. of CFU/ml of blood with the following procedure:		
		Vacutainer dilution	Isolation-10 centrifugation	Isolator-1.5 dil.
1	12 October		120	
	14 November	6,800	>300[a]	8,600
	15 November	3,800		3,200
	23 November			2,500
	29 November	3,900	8,500	7,700
	9 December	12,000	23,000	17,000
	21 December	4,600	24,000	13,000
2	16 December		>300	
	3 January		3,800	
	12 January	2,300	1,700	2,500
	17 January	2,400	2,900	2,800
	25 January	770	1,100	1,500
3	9 February		>300	
	10 February		>300	
	17 February	38	74	32
	24 February	158	183	
	12 March		686	

[a] When an early Isolator-10 count was >300, 10 and 100-fold dilutions of Isolator-10 sediment were prepared of all subsequent cultures.

TABLE 4. Antimicrobial susceptibility results of *M. avium* complex isolates from 35 patients with disseminated infection

Antimicrobial agent	Concn (μg/ml)	% Susceptible[a]
Isoniazid	0.2	0
	1	0
	5	30
Streptomycin	2	0
	10	22
Ethambutol	5	14
	7.5	76
	15	92
Ethionamide	5	30
	10	86
	15	89
Rifampin	1	0
	5	22
	10	35
Clofazimine	1	100
Cycloserine	30	100
	60	100
Ansamycin	2	100

[a] Isolates were considered susceptible to the antimicrobial agent if there was no growth or <1% growth on the test quadrant compared with countable colonies on the control quadrant.

remains to be studied. Acid-fast stains of feces have also been shown to be helpful in differentiating histopathology similar to Whipple's disease from *M. avium* complex infection of the intestine (Gold et al., ICAAC, abstr. no. 227). In the present study, when an acid-fast smear of feces was positive, the only acid-fast organisms that grew from culture were *M. avium* complex. Contamination of media by intestinal bacteria rarely occurred; thus the decontamination procedures described were adequate. Perhaps the normal intestinal flora of these patients is reduced because of their underlying disease, use of antibiotics, or other factors. The large number of mycobacteria excreted in the stool and the common bowel involvement suggest that the gastrointestinal tract may be the primary source of *M. avium* complex infection.

In the past, blood cultures for mycobacteria were infrequently performed in the microbiology laboratory. However, since the appearance of numerous disseminated *M. avium* complex infections in AIDS patients and the introduction of the new Isolator lysis blood culture systems, blood cultures for mycobacteria have become more common. In two recent reports (3, 12) and in this study, blood cultures were shown to be useful in detecting *M. avium* complex infection. In this study, blood cultures were a sensitive method of detecting disseminated disease; mycobacteria were detected in the blood of 96% of the patients with disseminated disease who had blood cultures taken. We have not detected these mycobacteria in the blood of AIDS patients who did not have known invasive mycobacterial disease.

The Isolator systems also provide the laboratory with a convenient method for determining the number of organisms in blood. Two recent reports described the value of Isolator quantitation in diagnosing and monitoring therapy in bacterial and fungal infections (1, 6). During our initial evaluation of the Isolator-7.5 system for recovery of bacteria and fungi (11), we found eight clear-cut cases where colony counts yielded information that was clinically useful (23). We recently described three AIDS cases where quantitative blood cultures were also useful in accessing *M. avium* complex infections and their treatment (B. Wong, T. E. Kiehn, F. F. Edwards, E. Whimbey, H. Donnelly, J. W. M. Gold, and D. Armstrong, Clin. Res. **31**:629A, 1983).

It is important that in vitro colony counts accurately reflect the magnitude of bacteremia: thus comparisons should be made between colony counts determined from unlysed blood specimens and the new lytic systems. The Isolator tubes contain the lytic agent saponin, which is used to lyse erythrocytes and thus permit more efficient recovery of microorganisms. Saponin also lyses leukocytes and should allow release of viable microorganisms. Recently an in vitro system for phagocytosis was used to demonstrate enhanced recovery of phagocytized *Staphylococcus epidermidis* from the Isolator-1.5 system when compared with unlysed blood (J. C. Richards and C. Bentsen, Abst. Annu. Meet. Am. Soc. Microbiol. 1984, C5, p. 237). These authors suggested that lysis and release of intracellular organisms may be critical for accurate quantitation of bacteremia. Lysis of leukocytes and release of mycobacteria by saponin may explain the increased numbers of mycobacteria the lytic systems recovered from our patient no. 1 (Table 2). We recently noted (the details will be reported elsewhere) that lysis of the leukocytes from that patient by either saponin or a conventional lytic system with desoxycholate allowed approximately a two- to fivefold increase in recovery of mycobacteria when these systems were compared with unlysed blood.

In the present study, colony counts of low numbers were easily enumerated with the Isolator-10 system. Where colony counts were higher, over 300 CFU/ml, they were more easily enumerated with 10- and 100-fold dilutions of blood from the Isolator-1.5 system. We now use the Isolator-10 to screen patients at high risk or who are suspected of having *M. avium* complex bacteremia, and the Isolator-1.5 is used for detecting and enumerating high-grade mycobacteremia.

Seventy-seven percent of the strains of mycobacteria from 26 of the patients with disseminated disease were serovar 4. No serovar 4 strain was isolated from eight patients with no evidence of invasive mycobacterial disease. These results contrast with other epidemiology reports (13, 19, 20). Before the 1950s and the advent of *M. avium* complex serotyping, the epidemiology of these organisms was difficult to study. Since the members of the *M. avium* complex, *M. avium* and *M. intracellulare*, cannot be differentiated by culture or biochemical methods, pathogenicity in chickens was initially used to differentiate between strains (5). Seroagglutination studies, begun by Schaefer in the 1950s (19), showed that there were three serovars of *M. avium*; they were designated 1, 2, and 3. Subsequently, 25 serovars of *M. intracellulare* have been described. Serovars 4, 5, 6, 8, and 9 exhibit virulence when injected intravenously into animals and have been designated as the "intermediate" group. Three reports have summarized the serovars of human *M. avium* complex isolates sent to National Jewish Hospital and Research Center, Denver, from 1960 to 1978. The first two reports showed that serotype 4 was not among the five most common isolates (19, 20). In descending order of frequency, the types were 1, 2, 9, 14, and 16 in the first study and 19, 1, 13, 9, and 16 in the second study. In the third survey, reported in 1981 for the years 1976 to 1978, the order of frequency was 8, 16, 4, 19, and 9; thus serovar 4 had then become the third most common isolate (13).

Ninety-eight isolates of *M. avium* complex from a variety of human diseases in Western Germany were serotyped (15). The most frequent isolates were serovars 2, 8, 9, and 1. Serovar 4 accounted for only 5% of the total isolates. This paper also reported the frequency of serotypes of 1,252 isolates from the tuberculous lesions of fowl and other animals, including cattle and pigs. Ninety-two percent of the mycobacteria were serovars 2, 3, and 8. Eleven strains of serovar 4 were isolated, and these were only from pigs. They suggested, however, that cattle and pigs were not usual reservoirs of human infection since in these animals granulomatous lesions were few, usually confined to lymph nodes, and excretion of bacilli in the feces usually did not occur. The finding that most of the strains from our disseminated cases were serovar 4 cannot readily be explained. However this observation suggests the posibility of a common source of infection. Eleven of 35 isolates, from AIDS patients, recently sent to National Jewish Hospital and Research Center from several areas of the United States other than New York City have also been serovar 4.

A second unusual characteristic of our 35 isolates from patients with disseminated disease was that 30 of them exhibited on primary isolation an intense yellow pigment, whereas only 1 of the 8 isolates from patients with noninvasive disease was a deep yellow. The remaining mycobacteria exhibited the more traditional buff color we usually associate with *M. avium* complex strains isolated in our laboratory. Both transparent and opaque morphologies were observed early in culture, and it was the opaque variety that became yellow. On agar media the yellow-pigmented *M. avium* complex in this study resemble other mycobacteria we

occasionally isolate. These other slow-growing mycobacteria also have a deep yellow pigment before exposure to light (scotochromogenic); however biochemically they differ from *M. avium* complex. They either have high catalase activity or produce urease, neither of which is produced by *M. avium* complex. These mycobacteria are grouped as the M.A.I.S. (*M. avium*, *intracellulare*, scrofulaceum) intermediates (9).

Thus, the pigmented organisms in this study represent a new observation in our laboratory, and these isolates are associated with disseminated disease, particularly in AIDS patients. However, all serovar 4 strains were not yellow, and not all yellow strains were serovar 4. There also was no obvious association between pigmentation, serotype, and a particular antimicrobial susceptibility pattern. It would be premature, at this time, to suggest that other serovars or colony types are not associated with disseminated disease. Isolates from a variety of patients, institutions, and geographic areas need to be studied.

The organisms were resistant to most of the antimicrobial agents to which *M. tuberculosis* is usually susceptible, and many were also resistant to several second-line agents. Our isolates were all susceptible to achievable serum levels of clofazimine, cycloserine, and ansamycin; however, whether these agents will be useful in vivo remains to be determined. Synergism was not investigated so far, although others have shown some promising results (E. J. Baron, O. G. W. Berlin, D. A. Bruckner, and L. S. Young, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 629, 1983).

Many of the isolates were resistant to concentrations of 5 μg of ethambutol and ethionamide per ml and susceptible to the next higher concentration tested. These results indicate that the in vitro minimum inhibitory concentration breakpoints were near these concentrations. Some laboratories do not routinely test mycobacteria to concentrations of 5 μg/ml, and indications of susceptibility at the next higher concentrations might be misleading. Such high serum concentrations of ethambutol and ethionamide may be difficult to achieve.

AIDS has thus presented clinicians and microbiologists with an infection with a well-known organism rarely seen in a disseminated form before, but now regularly seen in AIDS patients. Extraordinary features of this disease include bowel involvement with organisms frequently found in the stool and bacteremia with colony counts as high as 28,000 CFU/ml of blood.

We recommend for the diagnosis of *M. avium* complex infection in immunocompromised patients that the laboratory should receive stool for acid-fast smear and culture and blood for culture, in addition to the usual specimens such as urine, sputum, etc. In addition, a biopsy and acid-fast smear and culture should be performed on any organ, such as the gastrointestinal tract or liver, suspected of being infected. After treatment is started we recommend weekly acid-fast smears of stool and additional quantitative blood cultures.

ACKNOWLEDGMENTS

The assistance of the technologists of the Microbiology Laboratory, Memorial Sloan-Kettering Cancer Center, is gratefully acknowledged. Helen Rigopoulos helped prepare the manuscript. We thank Robert C. Good, Centers for Disease Control, Atlanta, and Leonid Heifets, National Jewish Hospital and Research Center, Denver, for confirming the antimicrobial susceptibility results of several of the bacterial isolates.

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