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Polyclonal *Mycobacterium avium* Infections in Patients with AIDS: Variations in Antimicrobial Susceptibilities of Different Strains of *M. avium* Isolated from the Same Patient

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Broth microdilution MICs were determined for pairs of strains isolated from five AIDS patients with polyclonal *Mycobacterium avium* infection. Four (80%) of the five patients were infected simultaneously with strains having different antimicrobial susceptibility patterns. These findings have implications for the interpretation of susceptibility data in *M. avium* prophylaxis and treatment trials.

Organisms of the *Mycobacterium avium* complex (MAC) are the most common cause of disseminated bacterial infection among patients with AIDS in the United States (10). Assessment of MAC treatment regimens has been based on both clinical and microbiologic responses, and attempts to correlate pretreatment antimicrobial susceptibility profiles with outcome have been made. These analyses have been complicated by the lack of a consensus on an optimal method for susceptibility testing of MAC. Various methods, including agar dilution, broth dilution, and radiometric broth testing, have been reported (16). The radiometric BACTEC method has been proposed as a standard (13).

Most studies have been unable to correlate outcome of MAC treatment with antibiotic susceptibility patterns (4, 14). In one retrospective study of MAC pulmonary infection, responders were more likely than nonresponders to have received antibiotics to which their isolates were susceptible (9). Two recent studies of AIDS patients with disseminated MAC infection treated with clarithromycin provide the first suggestion that outcome correlates with antimicrobial susceptibility: clinical and microbiologic responses were associated with clarithromycin MICs of ≤ 2 μg/ml, (2) and relapse was associated with MICs of ≥ 8 μg/ml (3).

The existence of polyclonal infection among AIDS patients with disseminated MAC infection is an additional factor which may need to be considered when antimicrobial susceptibilities are applied to treatment studies. Studies conducted by our group using nonradiometric culture techniques and pulsed-field gel electrophoresis (PFGE) have shown that as many as 24% of AIDS patients with disseminated MAC infection have polyclonal infection, that is, are infected simultaneously with two different strains of *M. avium* (1a, 15). In the present study we sought to determine whether different strains of *M. avium* isolated simultaneously from the same patient had different antimicrobial susceptibility patterns.

MAC was isolated from sputum, stool, bone marrow, liver, and Isolator (Wampole Laboratories, Cranbury, N.J.) blood cultures collected from human immunodeficiency virus-infected patients with CD4 counts of $<200/mm^3$ in Boston, Mass., and New Hampshire. For each site with a positive culture, three distinct colonies were subcultured and analyzed by PFGE according to previously published methods (1a, 15). Patients were defined as having monoclonal *M. avium* infection if all colonies from all positive body sites represented a single strain as resolved by PFGE analysis and polyclonal infection if the PFGE analysis resolved two or more distinct strains among the isolates available (1a, 15). Subcultures of isolates were characterized as transparent or opaque morphotypes (10). As previously noted, cultures of different morphotypes could be derived from the same strain (12) and had the same PFGE pattern (1, 1a).

Stock antibiotic solutions were prepared according to the manufacturer's directions from powders of defined potency and then diluted in water. Antibiotics from stock solutions were distributed in 100-μl amounts in serial twofold dilutions in sterile 96-well, round-bottom microtiter trays at two times the final concentrations to be tested.

Isolates were tested by a modification of previously described broth microdilution methods (5, 11). Isolates from frozen 7H9 broth stocks were plated as a confluent lawn on Middlebrook 7H10 agar plates. Lawns were harvested after 3 to 5 days and suspended in normal saline with 0.5% Tween 80. Single-cell suspensions were obtained by centrifugation at $50 \times g$ for 3 min. An aliquot of the supernatant was diluted with normal saline to an optical density at 540 nm of 0.1, representing $\sim 10^8$ CFU/ml, as determined by plating serial 10-fold dilutions on 7H10 agar plates and determining colony counts after 7 to 10 days. The bacterial suspension was diluted in 2× 7H9 broth with Middlebrook ADC enrichment (Becton Dickinson Microbiology Systems, Cockeysville, Md.) to a concentration of $\sim 1 \times 10^5$ CFU/ml, and 100 μl was distributed into microtiter wells containing drug as described above to a final inoculum of $\sim 5 \times 10^4$ CFU/ml. The 7H9 broth used to test clarithromycin and azithromycin was adjusted to pH 7.4 by addition of sodium hydroxide. Plates were read after 10 to 12 days of incubation at 37°C in an atmosphere containing 5% CO_2. Technicians reading the MIC results were not aware of PFGE results. The MIC was defined as the lowest concentration of drug at which there was no significant visible growth. Antibiotic susceptibility patterns were determined for each strain: patterns were considered different if MICs of at least two antimicrobial agents differed by >2 dilutions.

To determine reproducibility of MICs observed among different assays and among different colonial morphotypes of the

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TABLE 1. MICs for paired isolates from five AIDS patients with polyclonal *M. avium* infection

Patient-visit no.	Source-colony no.[a]	MIC [μg/ml] (resistant MIC) (reference) of[b]:							
		CLR (≥8.0) (3)	AZI (≥16.0) (7)	CF (≥0.5) (13)	AMK (≥8.0) (13)	EMB (≥8.0) (13)	CIP (≥4.0) (13)	SPA (≥1.0) (17)	RBN (≥0.5) (8)
1031-1[c]	Blood-1	2	4	≤0.12	4	16	0.5	1	≤0.06
	Feces-3	0.12	0.5	≤0.12	4	8	0.5	0.25	≤0.06
5026-1[c]	Blood-2	4	2	0.5	8	4	4	4	0.12
	Blood-3	≤0.06	0.5	0.25	2	8	0.25	0.25	≤0.06
5029-3[c]	Blood-1	4	4	0.5	16	8	16	8	0.5
	Bone Marrow-1	0.25	≤0.25	≤0.12	0.5	8	0.25	0.25	≤0.06
5060-1	Blood-3	0.25	≤0.25	≤0.12	4	4	1	1	≤0.06
	Feces-1	4	2	≤0.12	4	16	1	1	≤0.06
1057-2	Liver-2	0.12	0.5	≤0.12	4	4	0.25	0.25	≤0.06
	Liver-3	0.25	0.5	≤0.12	4	4	0.5	0.5	≤0.06

[a] For each patient, the two isolates represent different strains as determined by PFGE. Three colonies were selected from positive cultures from each body source and are numbered 1 to 3. For all patients except 1057, transparent morphotypes of both strains were tested; for patient 1057, opaque morphotypes were tested for both strains.

[b] CLR, clarithromycin (Abbott Laboratories); AZI, azithromycin (Charles Pfizer, Inc.); CF, clofazimine (Ciba-Geigy Corp.); AMK, amikacin (Bristol Laboratories); EMB, ethambutol (Lederle Laboratories); CIP, ciprofloxacin (Miles Laboratories); SPA, sparfloxacin (Parke-Davis); RBN, rifabutin (Adria Laboratories).

[c] Received prior antimycobacterial therapy (1031 received INH, rifampin, and pyrazinamide; 5026 received rifampin, CF, EMB, AMK, and CIP; 5029 received CLR, CLO, and EMB).

same strain, replicate determinations (median, 6; range, 2 to 16) were performed for seven strains from six patients. The range of MICs observed was >2 dilutions for 0 (0%) of 140 antibiotic-strain combinations assayed by using opaque morphotypes and 6 (2.5%) of 244 combinations assayed by using transparent morphotypes. For two strains, both transparent and opaque morphotypes were tested. The range of MICs observed was >2 dilutions for four of the eight antibiotics tested for one strain and for three antibiotics with the other strain. In each of these instances, the mean MICs for the opaque morphotypes were lower than those for the transparent variants of the same strain.

Isolates were available from five AIDS patients with polyclonal infection. Three of the five patients were receiving multiple-drug antimycobacterial therapy at the time that cultures demonstrated polyclonal infection. Antimicrobial susceptibilities were determined for five pairs of strains from the same patient; for each pair, the same colonial morphotype was tested for both strains (Table 1). For four of the five polyclonal infections, the two infecting strains had different susceptibility patterns as defined above. The observed MICs were compared with resistance breakpoints suggested by other investigators (3, 7, 8, 13, 17). For each of the eight antimicrobial agents tested, the number of patients simultaneously infected with one susceptible and one resistant isolate was as follows: clarithromycin and azithromycin, each zero of five; rifabutin, one of five; clofazimine, amikacin, ethambutol, and ciprofloxacin, each two of five; and sparfloxacin, three of five. Each of the three patients who had received prior antimycobacterial therapy was infected with one strain of *M. avium* that was resistant to one or more antimicrobial agents to which the other strain was susceptible.

We have demonstrated that AIDS patients with polyclonal *M. avium* infection may be infected simultaneously with strains that differ appreciably in their antimicrobial susceptibilities. Such differences likely reflect the wide variability in susceptibility patterns found among random isolates of MAC (7). Of potential significance, the differences between two infecting strains were most apparent for strains from patients who were receiving antimycobacterial treatment; susceptible and resistant strains of *M. avium* were present simultaneously in all three such patients.

Polyclonal infections can be detected only by separately isolating and analyzing multiple individual colonies from a primary culture. Consequently, the multiple sensitivity patterns identified in this study would not have been detected with standard microdilution MIC or radiometric broth susceptibility testing methods which are usually applied to subcultures derived from a single colony, a pool of three to five colonies, or a broth (6, 13). If single colonies are used for susceptibility testing, more-resistant coinfecting strains might be missed completely. If a pool of colonies or a broth subculture is used for susceptibility testing, the results may fail to reflect the most resistant of multiple coinfecting strains. In view of the uncertainties associated with susceptibility testing for MAC infection in general and for polyclonal MAC infection in particular, it is difficult to recommend routine susceptibility testing of primary clinical isolates at this time. Susceptibility testing should be considered for MAC isolates associated with treatment or prophylaxis failure.

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