Repeat Positive Cultures in *Mycobacterium intracellulare* Lung Disease after Macrolide Therapy Represent New Infections in Patients with Nodular Bronchiectasis

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The genomic DNA patterns (genotypes) of 55 episodes of late positive sputum isolates, collected after ⩾4 consecutive months of negative sputum cultures, in prospective macrolide treatment trials of *Mycobacterium avium* complex (MAC) lung disease were assessed by pulsed-field gel electrophoresis (PFGE). Having ⩾2 cultures positive for MAC after completion of therapy was documented 23 times; of 20 episodes studied by PFGE, 17 (85%) represented new genotypes (i.e., new infections), and 87% occurred in patients with nodular bronchiectasis. With ⩾2 positive cultures after therapy was stopped prematurely, 6 (86%) of 7 episodes were relapses. Single positive cultures after completion of therapy occurred 16 times; only 1 (6%) was predictive of a subsequent relapse. No late isolates were macrolide resistant. Thus, relapses of MAC lung disease with these macrolide regimens are unusual, and most infections after completing therapy resulted from new strains in patients with nodular bronchiectasis.

Treatment of *Mycobacterium avium* complex (MAC) lung disease has always been problematic. The introduction of the newer macrolides has improved the outlook for most patients, but many questions remain. These questions include the duration of drug therapy, the need for daily (vs. 3 times weekly) therapy, the number and choice of companion drugs, and what relapse rates can be expected with long-term (e.g., 5-year) follow-up. Single positive sputum cultures due to MAC that are not indicative of disease have long been recognized and presumably reflect transient infection from the environment or contamination. Because MAC are common in the environment, repeat or multiple infections are a risk in patients who have been treated successfully. Indeed, a recent study suggested that the average patient with nodular bronchiectasis is infected with 2 or 3 genotypes (i.e., isolates with different genomic large restriction-fragment patterns separated by pulsed-field gel electrophoresis [PFGE]) [1].

We have been involved in prospective macrolide treatment trials since 1992. As part of the evaluation of the success of therapy, we have performed genomic DNA strain comparisons of initial and subsequent late positive isolates after sputum conversion for all patients entered into the treatment trials. This study presents the interim results of these comparisons.

Patients, Materials, and Methods

Patient population. Patients with MAC lung disease were either diagnosed at the University of Texas Health Center (Tyler) or referred there because of the Center's expertise with therapy of that disease. They were then enrolled in 1 of 5 sequential MAC treatment trials. Patients with known or suspected HIV disease were excluded. Disease diagnosis was based on the diagnostic criteria of the American Thoracic Society [2]. Three pretreatment sputum cultures were obtained, and then cultures were obtained once a month while patients were receiving therapy. When patients were not receiving therapy, cultures were obtained monthly for the first 6 months, then every 3–6 months. Patients' chest radiographs and computerized axial tomography scans were reviewed, and each patient's disease was categorized as either nodular bronchiectasis or upper lobe fibrocavitary disease [1, 3, 4]. Patients were included in the treatment trials only if their pretreatment MAC isolate was macrolide susceptible. Sputum cultures were summarized every 1–2 months for each patient, and the results were screened for late positive cultures. The case summaries were reviewed for all patients as to the timing of cultures relative to their therapy. A single study coordinator monitored all patients in the studies since they began in 1992. Details of these treatment trials and preliminary results

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Informed consent was obtained from the patients or their parents or guardians, and human experimentation guidelines of the US Department of Health and Human Services and those of the authors' institutions were followed in the conduct of the clinical research. The study protocol was approved by the Human Subjects Institutional Review Board of the University of Texas Health Center at Tyler.

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are presented elsewhere [5–9]. Therapy included clarithromycin or azithromycin, rifampin or rifabutin, and ethambutol to be taken daily or 3 times weekly, until the patient was culture negative, while receiving therapy for 12 months. A few patients who were treated and monitored in the same manner but who did not enroll in the trials were included.

Cultures. Before the initiation of therapy or during the first few months of therapy, multiple cultures were obtained for MAC. Details of the sputum processing are presented elsewhere [1, 5, 6]. In brief, specimens were decontaminated with *N*-acetylcysteine and 2% sodium hydroxide and then plated on Middlebrook 7H10 agar and into a broth medium. Initially, we used a BACTEC 12B bottle (Becton-Dickinson) but later switched to the ESP system (Trek Diagnostics). For positive cultures, a cotton swab sweep of either the solid media or a subculture of the broth medium was added to trypticase soy broth with 15% glycerol and frozen at −70°C until needed. Most single-positive late cultures were taken from the broth medium bottles, which were saved for 24 months to be sure they were not needed. In early trials, cultures were considered to be positive only if the organism grew on solid medium (i.e., one culture growing in the broth medium while the patient was receiving therapy was not considered to be significant, and therapy was not altered). Subsequently, growth in either medium was interpreted as significant.

PFGE. Each patient's cultures were subjected to genomic DNA comparisons (while the patient was receiving therapy) if the patient's sputum converted to negative for a minimum of 4 months and the patient later had a single positive culture or, if the patient had any positive or early discontinuation cultures after the completion of therapy, as long as the patient was culture negative for at least 6 months during therapy. All available cultures were subjected to study by PFGE.

Patterns of large restriction fragments of genomic DNA were obtained by PFGE, as described elsewhere [10, 11]. In brief, organisms were incorporated into low-melting-point agarose plugs, then lysed with lysozyme (1 mg/mL), sodium dodecyl sulfate (1%), and proteinase K (1 mg/mL). DNA was digested with *Dra*I and *Xba*I and separated with a CHEF Mapper system (Bio-Rad Laboratories) at 14°C for 20 h at 6 V/cm. Pulse time was ramped from 3 to 12 s after *Xba*I digestion, from 5 to 15 s for 14 h, and then from 60 to 70 s for 6 h after *Dra*I digestion. Gels were photographed after staining with ethidium bromide.

Strains were compared for relatedness using the method of Tenover et al. [12], with minor modification specifically for MAC based on in-house experience with this method. Isolates were considered to be the same ("indistinguishable") if they showed no fragment differences with either restriction enzyme, closely related if they differed by 2–3 bands (i.e., restriction fragments) with either or

Table 1. Group 1 patients: consecutive negative cultures for ⩾10 months during therapy, followed by recurrences of multiple positive cultures after completion of therapy.

Macrolide regimen, patient	Age, years	Months culture negative		Pre/post strains studied	No. of late positive cultures	Results of PFGE	Results of MEE	Other potential pathogens recovered
		Receiving treatment	Not receiving treatment					
Azithromycin								
1[a]	86	11	1	3/2	4	New strain	New	None
2[a]	86	11	8	3/2	2	Relapse	ND	None
3	66	12	9	2/2	2	New strain	New	*P. aeruginosa*
4	52	12	10	1/11	18	New strain	New	*P. aeruginosa* and *M. kansasii*
5	62	13	14	9/4	6	New strain	New	None
6	74	13	20	10/4	5	New strain	New	*M. kansasii, P. aeruginosa,* and *M. abscessus*
7	67	12	32	3/2	3	New strain	New	None
8	54	12	36	1/2	2	New strain	New	None
9	33	12	40	3/4	4	New strains	ND	*P. aeruginosa* and *M. abscessus*
Clarithromycin								
10	54	12	2	10/3	5	New strain	New	*M. abscessus* and *P. aeruginosa*
11	86	12	3	1/2	2	New strain	New	*S. maltophilia*
12	69	16	3	0/0	3	No PFGE[b]	ND	None
13	69	12	3.5	5/2	2	Relapse	Relapse	None
14[b]	68	12	5	3/2	2	Possibly related	Relapse	*M. abscessus* and *P. aeruginosa*
15	64	13	10	4/2	2	New strains	ND	*P. aeruginosa*
16	73	12.5	15.5	2/5	8	New strain	New	None
17[b]	68	12	21	3/5	5	New strains	ND	*M. abscessus* and *P. aeruginosa*
18	57	12	26	0/0	2	No PFGE[b]	ND	Unknown
19	45	12	29	1/2	2	New strain	Relapse	*Klebsiella* species
20	61	12	31	0/5	7	No PFGE[b]	ND	*P. aeruginosa* and *M. abscessus*
21	54	12.5	39	2/2	3	New strain	New	None
22	63	12.5	48	3/4	4	New strain	ND	*M. fortuitum*
23	65	12.5	56	4/3	6	New strain	ND	*P. aeruginosa*
Total		283	462	73/70	99			13/22
Mean	63.9	12.3	20.0		4.3			

NOTE. MEE, multilocus enzyme electrophoresis; *M. abscessus, Mycobacterium abscessus; M. fortuitum, Mycobacterium fortuitum; M. kansasii, Mycobacterium kansasii;* ND, not determined; *P. aeruginosa, Pseudomonas aeruginosa;* PFGE, pulsed-field gel electrophoresis; pre, pretreatment or early therapy; post, late positive after therapy; *S. maltophilia, Stenotrophamonas maltophilia.*

[a] Same patient.

[b] Pretreatment isolate(s) not available for study.

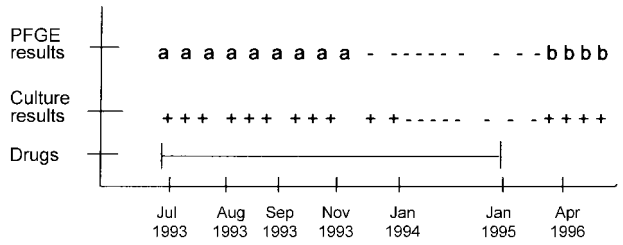

Figure 1. Culture and pulsed-field gel electrophoresis (PFGE) results over time in a patient with nodular bronchiectasis and repeat infection due to *Mycobacterium avium* complex (MAC) after initial successful drug therapy. The pattern of the first genotype is labeled "a," whereas the pattern of the second genotype is labeled "b." "Drugs" refers to MAC drug treatment. +, Positive culture; −, negative culture.

both enzymes, possibly related if they differed by 4–6 bands but >50% of well-resolved bands were the same with either or both enzyme, and different or unrelated strains if ⩾7 bands were different and <50% of well-resolved bands were the same with both enzymes. Relapse strains were those strains classified as indistinguishable from or closely related to pretreatment isolates, whereas new strains were strains classified as different or unrelated to the pretreatment strains. Possibly related strains were classified as possibly related.

Multilocus enzyme electrophoresis (MEE). Examples of each PFGE genotype from most patients who became infected with a new genotype after therapy were also subjected to MEE. Ten enzymes known to have multiple alleles for MAC were studied as described elsewhere [13]. Enzymes included 6-phosphogluconate deydrogenase (NADP coenzyme), malate dehydrogenase, leucine aminopeptidase, benzyl alcohol dehydrogenase, esterase, phosphoglucose isomerase, phosphoglucomutase, glutamate oxalacetic transaminase, adenylate kinase, and 6-phosphogluconate dehydrogenase (NAD coenzyme). By MEE, isolates were considered to be the same ("indistinguishable") if they had the same allelic pattern, possibly related if they differed by 1 closely related pattern, and different or unrelated if they differed by ⩾2 or more alleles or 1 very different allelic pattern.

Species identification. Initial and late positive cultures were confirmed as MAC by using the commercial DNA-RNA probe (Accuprobe; Gen-Probe). They then were identified as *M. avium* or *M. intracellulare,* by either the species-specific DNA-RNA probe (Accuprobe) or polymerase chain reaction (PCR) restriction analysis of the 439-bp [14] fragment of the 65 kDa *hsp* gene, as described elsewhere [14]. One isolate of each PFGE pattern was studied.

Susceptibility testing. Initial and selected late positive cultures were tested for susceptibility to clarithromycin using a broth microdilution system according to recently proposed NCCLS (formerly the National Committee for Clinical Laboratory Standards) guidelines [15]. Resistance was defined as an MIC of ⩾32 μg/mL, whereas susceptibility was defined as an MIC of ⩽16 μg/mL. Details of the method are provided elsewhere [5, 16].

Results

Patients and cultures. In total, 333 patients were enrolled (intent-to-treat) in the 5 macrolide treatment trials; 204 patients (61%) were enrolled to receive clarithromycin regimens, and 129 (39%) were enrolled to receive azithromycin regimens. All but 2 patients are currently not receiving study drugs or have completed therapy, but only ~20% have been not been receiving therapy for 5 years. Thus far, 51 patients have had 55 episodes of ⩾1 late positive culture for MAC. A total of 296 cultures from these patients was submitted for study by PFGE, a mean of 5.4 cultures per patient episode.

Patients were divided into 4 groups. Group 1 patients had consecutive negative cultures (in both the liquid and solid medium) for ⩾10 months during therapy and multiple (⩾2) positive cultures after completion of drug therapy. (The late positive cultures were collected within 6 months of each other.) Group 2 patients had consecutive negative cultures for ⩾6 to <10 months during therapy and multiple (⩾2) positive cultures collected within a 6-month period after premature discontinuation of drug therapy; patients who were culture negative for <6 months before discontinuing therapy were not evaluated. Group 3 patients had a single positive culture while receiving drug treatment, after having negative sputum cultures for at least 4 consecutive months and no other positive cultures in the ensuing 3 months. Group 4 patients had consecutive negative cultures for ⩾10 months during therapy and a single positive culture after satisfactory completion of drug therapy, with no other positive cultures in the next 6 months after the single positive culture.

PFGE. All isolates gave satisfactory large restriction-fragment patterns for analysis. Group 1 isolates caused 23 episodes in 21 patients (detailed in table 1). The mean number of follow-up cultures (not during therapy) for the 22 episodes with detailed follow-up was 21.5 (range, 2–65 cultures). Eighteen (82%) of these 22 episodes were in patients with ⩾10 follow-up cultures. Nine patients (39%) had 2 positive cultures, 3 patients (13%) had 3 positive cultures, and 11 patients (48%) had ⩾4 positive cultures.

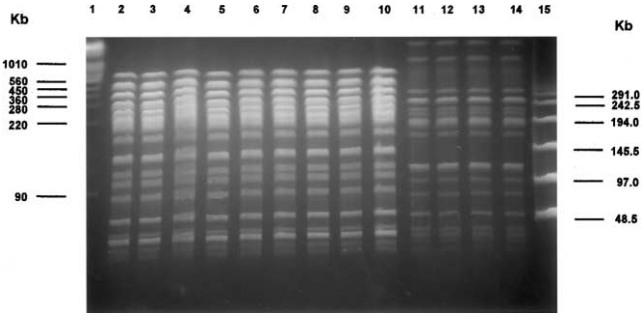

Figure 2. *Dra*I large restriction-fragment patterns of genomic DNA of *Mycobacterium avium* complex (MAC) isolates from a patient with nodular bronchiectasis and second MAC infection shown in figure 1. *Lane 1,* yeast DNA standards; *lanes 2–10,* identical patterns from 9 isolates collected pretreatment or during initial therapy between July and November 1993; *lanes 11–14,* identical patterns but different from early ones from 4 isolates collected between April and June 1996; *lane 15,* lambda DNA standards.

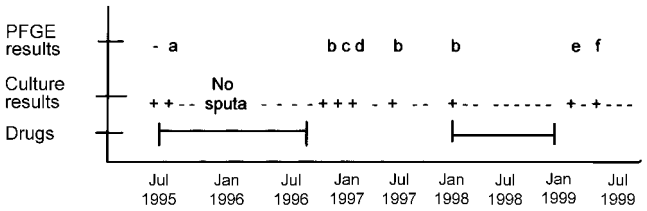

Figure 3. Culture and pulsed-field gel electrophoresis (PFGE) results over a 4-year period in a 65-year-old male with nodular bronchiectasis. Each letter (a–f) represents a different PFGE type. "Drugs" refers to *Mycobacterium avium* complex drug treatment. +, Positive culture; −, negative culture.

Of the 23 episodes, 20 had PFGE comparison of both pretreatment isolates (mean, 3.7 isolates) and posttreatment isolates (mean, 3.0 isolates). Of these 20 isolates, 17 (85%) involved ⩾1 new strain, 1 (5%) involved a possibly related strain, and 2 (10%) involved relapse strains. For the remaining 3 episodes, no pretreatment isolate was available for comparison.

The mean time to infection in the 14 episodes of new infections in patients with nodular bronchiectasis was 24.2 months. One patient had 2 separate episodes with different strains 5 and 21 months after stopping therapy, and another had 2 episodes 1 and 8 months after therapy. Overall, of the 23 episodes of multiple positive cultures after completion of an acceptable course of therapy, only 1 (4.3%) was shown to be a definite relapse. Examples of the time course, culture results, and PFGE in 2 patients with late reinfection is shown in figures 1–4.

Twenty (87%) of the 23 episodes occurred in patients with underlying nodular bronchiectasis, including both patients with 2 separate episodes. Of the 18 patients with nodular bronchiectatic lung disease, 14 (78%) were women, none was a current smoker, 10 (55%) had never smoked, and none abused alcohol. In contrast, of the 3 patients with upper lobe cavitary disease, all 3 were men, 2 were current smokers, all had smoked >50 pack-years, and 2 abused alcohol. These findings are considered to be typical of these 2 patient populations with MAC [1].

Group 2 isolates were from 7 patients who had stopped drug therapy but had stopped prematurely, such that they were culture negative for ⩾6 but <10 months during therapy (table 2). PFGE was performed on isolates from all patients. Of these 7 patients with multiple positive cultures, 6 (86%) had relapses with the same genotype (figure 5).

Group 3 consisted of 8 isolates from 8 patients who had single positive cultures after sputum conversion of at least 4 consecutive months, but while still receiving therapy, and 2 isolates (4 months apart) from a ninth patient (table 3). These were the only positive results the patients had after sputum conversion during therapy. All 10 cultures were acid-fast bacilli (AFB) smear negative. Five (50%) were positive only in the broth culture medium, and 4 (40%) were positive in the liquid medium with <20 colonies on Middlebrook 7H10 agar (table 3). PFGE was performed on 9 of 10 of the cultures. In 5 patients, the single positive culture was the same as the pretreatment strain (genotype), and all 4 patients (100%) in whom this single positive culture was disregarded and received a standard course of therapy (culture negative for 10–12 months, except for the 1 positive broth culture) experienced relapse within 3 months of stopping therapy. Four single positive isolates from 3 patients had a different pattern (different from the pretreatment PFGE type), and none of the 3 patients experienced relapse or had additional positives. The last patient who had a single positive culture at 6 months but whose isolate was not saved and who was culture negative only 8 months (the single positive was disregarded), experienced relapse within 3 months of stopping therapy. Of the 9 patients, 7 had underlying nodular bronchiectasis. Thus, overall, 50% of single positives were pretreatment genotypes and were predictive of relapse, whereas 40% of the cultures were new genotypes that had no apparent impact on the outcome of therapy.

Group 4 consisted of isolates from 15 single positive MAC cultures (with no other positive cultures in the ensuing 6 months) from 14 patients who had completed therapy, with ⩾10 months of consecutive negative cultures during therapy. Eleven (79%) of 14 isolates with quantitated cultures were recovered from the broth medium only. Of the 12 isolates studied by PFGE, 7 (58%) of 12 were new isolates, 2 (17%) of 12 were relapse isolates, 2 (17%) of 12 had possible related isolates, and 1 culture was identified as a laboratory contaminant. A second isolate was considered to be a laboratory contaminant but was not saved for PFGE. All but 1 of the 15 single positive cultures were recovered 3 or more months after completion of therapy, and the majority (75%) occurred >6 months after stopping therapy. Thus, single positive cultures were seen in patients after completion of therapy, and in only 1 patient (7%) was it predictive of a later relapse with the same strain. Isolates in Group

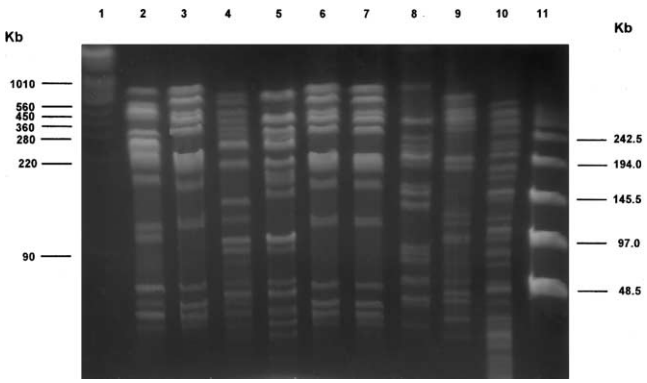

Figure 4. *Dra*I large restriction-fragment patterns of genomic DNA of *Mycobacterium avium* complex isolates from patient shown in figure 3. *Lane 1,* yeast DNA standards. *Lanes 2–9,* pulsed-field gel electrophoresis (PFGE) patterns from isolates collected from August 1995 to July 1999 in chronological order (labeled in figure 3 as a–f). PFGE patterns in lanes 3, 6, and 7 were the same (indistinguishable). All others were different. One additional isolate was obtained after July 1999 (not shown in figure 3) and it exhibited another pattern (pattern g, *lane 10*). *Lane 11,* lambda DNA standards.

Table 2. Group 2 patients: consecutive negative cultures (including broth) for ⩾6 but <10 months during therapy, followed by multiple positive cultures after stopping therapy prematurely.

Patient	Months culture negative: Receiving treatment	Months culture negative: Not receiving treatment	Pre/post strains studied	Clarithromycin MIC of late positive culture	Results of PFGE
1	9.5	1	2/3	S	Relapse
2	6	1	6/2	S	Relapse
3	9	2	1/5	S	New strains
4	8	2	5/1	S	Relapse
5	7	2.5	3/2	ND	Relapse
6	9	5.5	6/3	S	Relapse
7	9	5.5	1/2	S	Relapse
Total	57.5	19.5	24/18		
Mean	8.1	2.8			

NOTE. ND, not determined; PFGE, pulsed-field gel electrophoresis; pre, pretreatment or early therapy; post, late positive after therapy; S, susceptible.

4 are summarized in table 4, with comparison of restriction fragment–length polymorphism analysis patterns by PFGE in figure 6.

MEE. MEE was performed on 14 of the PFGE types from group 1 patients who had multiple positive cultures after completing recommended therapy. It was in agreement with PFGE with 12 (86%) of 14 of the episodes. It identified the 1 possibly related strain (by PFGE) as the same as the pretreatment strain, and identified a second strain defined as new by PFGE as the same as the pretreatment strain. Technical problems with the gels prevented analysis of the 6 additional paired strains (table 1).

Species identification. All PFGE types were confirmed as being MAC by use of the commercial RNA-DNA probe. Identification to species was performed on PFGE types in patients in group 1 with multiple positive cultures after completion of the recommended therapy. A total of 19 pretreatment isolates was studied, of which 18 (95%) were *M. intracellulare*. A total of 27 late positive PFGE types was studied, of which 24 (89%) were *M. intracellulare*. Overall, 91% of the 46 PFGE types were *M. intracellulare*.

Susceptibility testing. None of the new infection or relapse isolates was clarithromycin resistant. All pretreatment isolates of the patients with late positive cultures were also clarithromycin susceptible.

Discussion

Several important observations were apparent from the current study. One of the most important is documentation that reinfection after successful drug therapy occurs with MAC lung disease, but primarily (~90%) in patients with nodular bronchiectasis. This result is perhaps not surprising, since a previous PFGE study [1] showed that most patients with nodular bronchiectasis were infected with multiple genotypes, presumably a result of multiple infections. Reinfection was observed in only 3 patients with cavitary upper lobe disease, and this same previous PFGE study showed that patients with this form of disease are infected with a single strain [1].

Another observation is that relapse is rare in patients who are culture negative (both broth and solid media) for ⩾10 consecutive months while receiving a 3-drug, macrolide-containing regimen, with 85% of subsequent infections associated with multiple positive sputum cultures in this setting representing a new infection. In contrast, the majority of infections (86%) identified in patients who were culture negative for <10 consecutive months during therapy were relapses, rather than new infections. All the relapse isolates identified to date occurred within 10 months of stopping therapy. Relapse isolates were seen in 4 cultures. Three of these cultures were AFB smear negative, with positive cultures in the broth only, and were not followed by any subsequent positive cultures. The fourth patient was AFB smear positive with ⩾4 heavily positive cultures. Perhaps equally surprising, none of the relapse isolates tested was macrolide resistant. Because the current study includes patients from multiple protocols [5–9], only 20% of patients have been monitored for 5 years after discontinuation of treatment, so some changes in these percentages may occur.

Another important observation was the significance of a single positive culture of the infecting strain during the therapy period after sputum conversion. When this culture (usually a liquid medium bottle only with no growth on the solid media) was ignored, all patients subsequently experienced relapse. One patient who received another 12 months of negative cultures after her single positive has remained negative (not during therapy) for her infecting strain for >2 years. This also points out

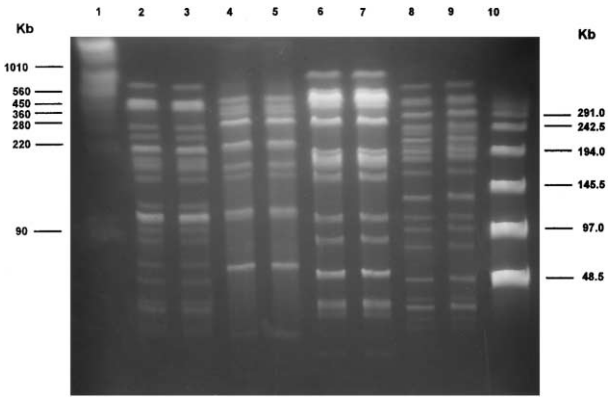

Figure 5. *Dra*I large restriction-fragment patterns of genomic DNA of *Mycobacterium avium* complex isolates from patients shown in table 2 who had consecutive negative cultures for ⩾6 but <10 months and then multiple positive cultures after stopping therapy prematurely. *Lane 1,* yeast DNA standards; *lanes 2* and *3,* patient 1 (relapse); *lanes 4* and *5,* patient 5 (relapse); *lanes 6* and *7,* patient 6 (relapse); *lanes 8* and *9,* patient 7 (relapse). The first isolate of each patient was from pretreatment or early therapy, and the second one was from a late positive culture obtained after stopping therapy prematurely. The 2 isolates of each patient had identical pulsed-field gel electrophoresis patterns. *Lane 10,* lambda DNA standards.

Table 3. Group 3 patients: consecutive negative cultures for >4 months, followed by a single positive culture, while still receiving therapy.

Patient	Months culture negative during therapy	PFGE results	Culture results	Outcome	No. of isolates tested by PFGE
1	4	Relapse	Broth only	Negative for next 6 mos. while receiving Rx; relapsed 1 mo. after Rx discontinued	8
2	5	Relapse	Broth (11 colonies)	Negative for next 7 mos. while receiving Rx; relapsed after Rx discontinued	6
3	5	Relapse	Broth only	Negative for next 12 mos. while receiving Rx; has not received Rx for 6 mos., without relapse	3
4	5.5	Relapse	Broth (1 colony)	Negative for next 2.5 mos. while receiving Rx; relapsed after Rx discontinued	8
5	6	New	No broth (6 colonies)	Remained negative until died while not receiving Rx	3
6[a]	6	New	Broth (1–2 positive cultures grown on agar)	Remained negative; has not received Rx for 2.5 years	6
7	6	ND	Broth (18 colonies)	Negative for next 2 mos. while receiving Rx; relapsed 3 mos. after Rx discontinued[b]	10
8	7	New	Broth only	Same as prior processed laboratory culture sample	4
9[a]	10	New	Broth only	Remained negative until Rx discontinued; has not received Rx for 2.5 years	5
10	10	Relapse	Broth only	Negative for next 2 mos. while receiving Rx; relapsed 2 weeks after Rx discontinued	15
Total					68

NOTE. All the patients in this group had nodular bronchiectasis. Mo, month; ND, not done; PFGE, pulsed-field gel electrophoresis; Rx, treatment.

[a] Same patient.

[b] This patient had poor compliance, with 2 degrees of intolerance.

the importance of using liquid media (broth) in patient follow-up during therapy and the need to do routine cultures after sputum conversion.

An important issue raised by these observations is whether DNA strain comparison is needed to make quality decisions about therapy of MAC lung disease. Treatment of MAC lung disease requires a major investment in time (mean, 16–18 months of therapy), money (the average drug cost alone is >$7,000 for a course of therapy), and family and health care provider commitment (e.g., ensuring the drugs are taken, avoiding toxicity, and getting to physician appointments). We believe that the availability of methods for strain comparison, such as PFGE, in patients with nodular bronchiectasis is helpful, if not essential. Several methods are available, including PFGE, MEE, arbitrarily primed–PCR, and (for *M. avium*) hybridization of repetitive elements [10, 17–22]. We recommend that at least 1 pretreatment isolate from all patients with MAC should be saved for at least 3 years from the start of therapy and that this isolate be compared to any subsequent positive cultures that may affect treatment.

In contrast, a single positive culture that was AFB smear negative and of low positivity (<10 colonies on solid media and/or positive in the liquid medium) after successful clinical therapy (with ⩾10 months of consecutive negative cultures during therapy) is generally not the same as the original infecting strain (only 15% of current cases), and is not a predictor of relapse. This presumes that subsequent cultures over a 6-month period after the single positive are negative.

Several features of the reinfections are disturbing. One is that they were often polymicrobial (i.e., involved multiple genotypes); 7 (44%) of 16 of those studied were polymicrobial. One would have expected a single strain or genotype to have been

Table 4. Group 4 patients: negative cultures for ⩾10 months during therapy, followed by a single positive culture after discontinuing therapy, with no other positive cultures within 6 months after the positive culture.

Patient	Months culture negative while not receiving therapy	Culture positive	PFGE result	Nodular bronchiectasis	Outcome (date of last culture)	No. tested Pre/Post PFGE
1	1.5	Broth only	New	+	Remained culture negative until death	1/1
2	3	⩾4 Grown on agar	ND (presumed laboratory contaminant)	−	Remains culture negative 26 mos. later (Sep 2000)	0/0
3	5.5	Broth only	New	+	Remained culture negative for 3 yrs.; relapsed with another strain	2/1
4	6	Broth only	Relapse	+	Remains culture negative 9 mos. later (Mar 2000)	1/1
5	7	Broth only	New	−	Remains culture negative 5 yrs. later (Jul 1999)	5/1
6	8	Broth only	Possibly related[a]	+	Remains culture negative 15 mos. later (Sep 2001)	1/1
7	10	Broth only	Relapse	+	Remains culture negative 25 mos. later (Aug 2001)	1/1
8	13	Broth only	New	+	Remains culture negative 4 yrs. later (Feb 2001)	1/1
9	14.5	Unknown (outside laboratory)	ND (no pre-Rx isolate)	+	Had multiple positive cultures, same as the single positive culture, 16 mos. later	0/5
10	16	Broth only	ND (lost)	+	Remains culture negative 32 mos. later (Feb 2001)	0/0
11	19	Broth only	Possibly related[b]	+	Remains culture negative 19 mos. later (Oct 2001)	1/1
12	23	Broth only	New	−	Remains culture negative 3.5 yrs. later (Jul 1999)	2/1
13	25	Broth only	New	−	Remains culture negative 3.5 yrs. later (Jul 1999)	5/1
14	30	Broth (6 colonies)	New	+	Remained culture negative until death	3/1
15	34	Broth (1 colony)	Laboratory contaminant	+	Remains culture negative 3.3 yrs. later (Oct 1999)	3/1
Total						26/17
Mean	15.1					

NOTE. All acid-fast bacilli smears were negative for this group of patients. Mos., months; ND, not done; post, late positive culture after therapy; pre, pretreatment or early therapy; Rx, treatment; yrs., years; +, positive; −, negative.

[a] Six bands' difference with *Xba*I, and 5 bands' difference with *Dra*I.

[b] Five bands' difference from each enzyme.

identified. In 2 patients, however, 2 distinct episodes involving different strains were identified (table 1). Did multiple genotypes infect the patient at the same time, or was the timing of cultures such that ⩾1 infection episode was missed? The observation that many episodes of positive cultures may be followed by periods of negative cultures suggests that the latter possibility is more likely.

The second surprising feature is how close the new infections in patients with nodular bronchiectasis occurred after stopping therapy, with a mean of only 24 months. This might support the concept that they were present all along in the patient, but just not active. Yet, true relapses of the identified original strains or genotypes were rare and all occurred within the first year after stopping therapy. It is most likely, in our opinion, that MAC infection is not the primary disease in patients with nodular bronchiectasis. The patients' immune-compromising disease that puts them at risk for MAC (i.e., the bronchiectasis) is unaltered by therapy, and continued environmental exposure may make reinfections only a matter of time for most patients with nodular bronchiectasis. The short time to reinfection could be a partial function of the length of follow-up, as only 20% of patients in these 5 treatment trials have been monitored (not during therapy) for at least 5 years. We suspect that reinfections in patients with nodular bronchiectasis will continue to be seen and will not just be clustered close to the time that therapy was stopped. Longer follow-up appears to be most important for patients with upper lobe fibrocavitary disease, where relapses occurred a mean of 4 years after stopping therapy. Infections due to MAC are a well recognized complication of prior granulomatous disease, especially following tuberculosis (so called "post-tuberculous infections"), but generally occur many years after the primary infection. It seems likely that more reinfections of this type will be seen, but may require many years more follow-up.

The other potential pathogens seen in these patients, primarily *Pseudomonas aeruginosa* and *Mycobacterium abscessus,*

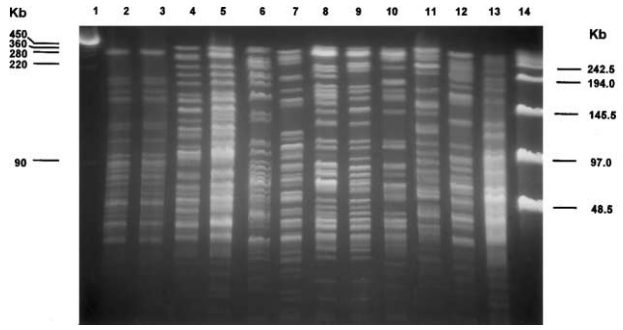

Figure 6. *Xba*I large restriction-fragment patterns of genomic DNA of *Mycobacterium avium* complex isolates from selected patients shown in table 4 who had negative cultures for ⩾10 months during therapy and then had single positive cultures while not receiving therapy. *Lane 1,* yeast DNA standards; *lanes 2* and *3,* patient 4 (relapse); *lanes 4* and *5,* patient 6 (possibly related); *lanes 6* and *7,* patient 8 (new); *lanes 8* and *9,* patient 11 (possibly related); *lanes 10* and *11,* patient 12 (new); *lanes 12* and *13,* patient 14 (new). The first isolate of each patient was from pretreatment or early therapy, and the second one was from a late positive culture after negative cultures for ⩾10 months during therapy. *Lane 14,* lambda DNA standards.

bear a striking resemblance to the microbial flora seen in some patients with cystic fibrosis (CF). No genetic defect has been described in these patients with nodular bronchiectasis as it has in patients with CF, but it is a strong possibility. The most logical common denominator showed by the 2 groups would seem to be their bronchiectasis, but more studies are needed to help address this issue.

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