

## BRIEF REPORT: RIFAMPIN-RESISTANT TUBERCULOSIS IN A PATIENT RECEIVING RIFABUTIN PROPHYLAXIS

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**D**RUG-RESISTANT tuberculosis has become a major public health problem in the United States.<sup>1,2</sup> In 1979 less than 1 percent of tuberculosis isolates from untreated patients in New York City were resistant to rifampin, as compared with 9 percent in 1991.<sup>3</sup> Rifampin-resistant tuberculosis is a serious threat because responses to therapy are more difficult to achieve and require longer courses of treatment.<sup>4-8</sup>

Rifabutin is a derivative of rifamycin S that is recommended and widely used as prophylaxis against *Mycobacterium avium* complex infection in patients infected with the human immunodeficiency virus (HIV) who have low CD4 lymphocyte counts.<sup>9,10</sup> Since rifabutin was introduced in 1993, concern has been expressed about its cross-activity against *M. tuberculosis* and the possibility that prophylactic use of the agent might induce drug-resistant tuberculosis.<sup>11</sup> A single case of rifampin-resistant tuberculosis has been reported in a patient taking rifabutin prophylactically.<sup>12</sup>

In this report we describe a patient with the acquired immunodeficiency syndrome (AIDS) and fully susceptible *M. tuberculosis* disease who was treated with directly observed therapy followed by rifabutin prophylaxis, who then had a relapse of tuberculosis caused by a rifampin-resistant strain that was otherwise indistinguishable from the initial infecting organism.

### CASE REPORT

A 35-year-old man with HIV infection was hospitalized with a two-month history of fever, night sweats, cough, dyspnea, and an 18-kg weight loss. He had a history of thrush and peripheral neuropathy, and his CD4 lymphocyte count was 7 per cubic millimeter. He was taking fluconazole and nortriptyline. A chest radiograph revealed bilateral hilar adenopathy without infiltrates. A sputum sample showed many acid-fast bacilli, and culture of the sputum sample yielded

*M. tuberculosis* susceptible to isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin. The patient was given isoniazid, rifampin, pyrazinamide, and ethambutol daily for 16 days and then twice weekly for 6 weeks, followed by isoniazid and rifampin twice weekly for 33 doses over a 5-month period. All therapy was directly observed by a nurse.<sup>13</sup> After one month smears and cultures of the patient's sputum remained positive for *M. tuberculosis*, but they became negative after two months of therapy.

After five months pneumonia with diffuse infiltrates developed that resolved with empirical treatment with trimethoprim and sulfamethoxazole; smears and cultures of the sputum were negative for mycobacteria. A chest radiograph obtained nine months after the diagnosis of tuberculosis was normal. Ten months after the diagnosis smears and cultures of a sputum sample were negative for mycobacteria. At this time the patient saw a private physician and began taking 300 mg of rifabutin daily as prophylaxis against *M. avium* complex infection.

Two months later — one year after the initial diagnosis of tuberculosis — fever, a productive cough, and severe dyspnea developed. A chest radiograph showed diffuse interstitial infiltrates. The patient was taking rifabutin, fluconazole, and nortriptyline. Rifabutin was stopped two days after admission. A smear of bronchoalveolar-lavage fluid was negative for acid-fast bacilli and *Pneumocystis carinii*. The patient was given a combination of trimethoprim and sulfamethoxazole and broad-spectrum antibiotics but died of respiratory failure after 10 days. A strain of *M. tuberculosis* resistant to rifampin and rifabutin but susceptible to isoniazid, pyrazinamide, ethambutol, and streptomycin subsequently grew in cultures of bronchoalveolar-lavage fluid and blood.

### METHODS

The susceptibility of *M. tuberculosis* isolates to rifampin, rifabutin, and other drugs was determined by the BACTEC radiometric culture method<sup>14</sup> and the modified proportion method.<sup>15</sup> Analysis involving restriction-fragment-length polymorphisms (RFLPs) was performed. Chromosomal DNA from *M. tuberculosis* was extracted, digested with *PvuII*, and hybridized by Southern blot techniques with a 245-bp portion of the IS6110 repeat element labeled with phosphorus-32.<sup>16,17</sup> Alternatively, chromosomal DNA from *M. tuberculosis* was extracted, digested with *AluI*, and hybridized by Southern blot techniques with <sup>32</sup>P-labeled pTBN12 containing the *M. tuberculosis* polymorphic guanine-cytosine (GC)-rich repetitive sequence.<sup>18,19</sup> A segment of the RNA polymerase  $\beta$  subunit (*rpoB*) gene from *M. tuberculosis* chromosomal DNA was amplified with the polymerase chain reaction (PCR) with the use of primers that amplify a 411-bp product<sup>20</sup>; *TaqI* polymerase was used in standard reaction mixes for 30 cycles. A biologic safety cabinet and aerosol tips were used to set up solitary PCRs for each isolate on separate days, and each amplification was accompanied by a control reaction in which the DNA template was omitted. After gel purification, both strands of the PCR products were sequenced according to the dye-terminator method. Amplification and sequencing of the *rpoB* segment were performed twice for each isolate.

### RESULTS

Analysis of the *M. tuberculosis* isolates from the patient's first and second episodes of tuberculosis showed that the strains were identical except in their susceptibility to rifampin and rifabutin and in their *rpoB* sequences. DNA fingerprinting by RFLP analysis with *PvuII* and IS6110 revealed an identical two-banded pattern in the two isolates (Fig. 1), suggesting a common clonal origin. Because of the limited ability of RFLP analysis to differentiate between strains when the number of IS6110 bands is small, we repeated the test using *AluI* and the polymorphic GC-rich repetitive sequence as a probe.<sup>19</sup> This analysis revealed identical 10-banded patterns in each isolate (Fig. 1), confirming

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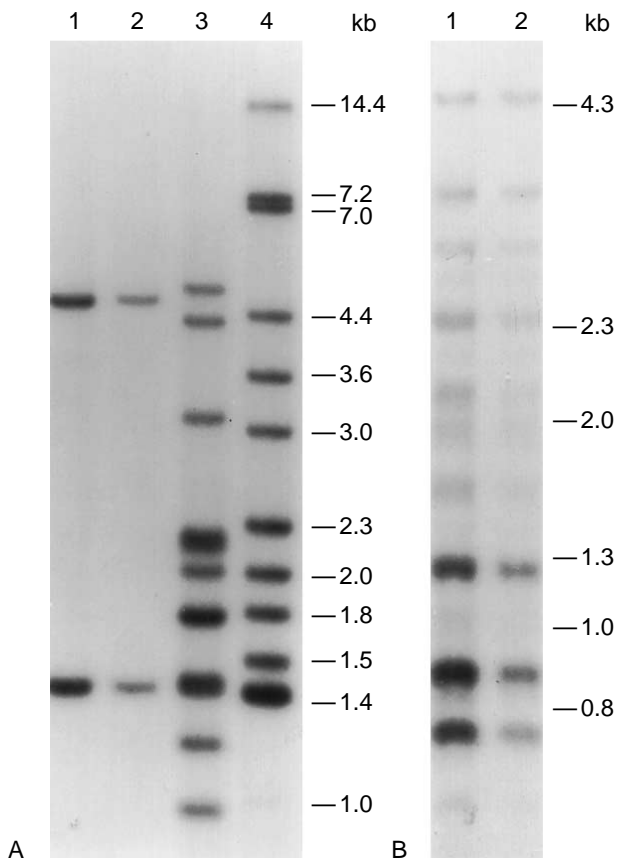


Figure 1. Results of RFLP Analysis of *M. tuberculosis* Chromosomal DNA.

Panel A shows the results of Southern blotting after RFLP analysis with *PvuII* and *IS6110*. Lane 1 shows an isolate obtained during the patient's first episode of tuberculosis; lane 2, an isolate obtained during the second episode, 10 days before his death; lane 3, an unrelated control strain; and lane 4, reference strain Mt14323.<sup>17</sup> The numbers on the right indicate the band sizes inferred from the Mt14323 markers. Panel B shows the results of Southern blotting after RFLP analysis with *AluI* and the polymorphic GC-rich repetitive sequence of *M. tuberculosis*. Lane 1 shows an isolate obtained during the patient's first episode of tuberculosis, and lane 2 an isolate obtained during the second episode, 10 days before his death. The numbers on the right indicate the band sizes and were determined from *HindIII*-digested coliphage lambda DNA markers (not shown), which were visualized by stripping the blot and reprobing with labeled lambda DNA.

the probable clonal origin of the two isolates. Drug-susceptibility testing of the two *M. tuberculosis* isolates, however, revealed that the original was susceptible to all drugs tested, whereas the strain isolated during the relapse was resistant to rifampin and rifabutin.

Amplification and sequencing of a segment of the *rpoB* associated with resistance to rifampin in *M. tuberculosis* and other bacteria<sup>21-23</sup> revealed a mutation (a change from TCG to TTG) resulting in the substitution of a leucine residue for serine at position 531 in the second isolate. This was the most common mutation iden-

tified in a recent study of 66 clinical isolates of rifampin-resistant *M. tuberculosis*.<sup>20</sup>

To investigate whether our patient might have been reinfected with an *M. tuberculosis* strain prevalent in Baltimore, we reviewed our data base of RFLP patterns in 101 isolates for the past two years.<sup>24</sup> Strains with matching RFLP patterns on analysis with *PvuII* and *IS6110* or *AluI* and the polymorphic GC-rich repetitive sequence have been identified in only two other cases. Both were diagnosed within four months of our patient's first illness, and both isolates were susceptible to rifampin. No rifampin-resistant isolates with the same RFLP patterns were identified, and no other isolates of this strain were found in the period after our patient completed treatment. Despite an extensive investigation of the patient's contacts, we were unable to establish an epidemiologic link between our patient and the other patients with rifampin-susceptible *M. tuberculosis* with the same DNA fingerprint.

## DISCUSSION

We report a relapse of tuberculosis characterized by resistance to rifampin and rifabutin after exposure to both drugs in a patient whose infection was initially drug-susceptible. The matching RFLP patterns of the two *M. tuberculosis* isolates indicate that the strains were virtually identical. The small number of other cases of tuberculosis caused by this strain in the community at the time of the relapse makes it highly unlikely that the patient was reinfected with either a drug-susceptible or a drug-resistant *M. tuberculosis* variant of the same clone.<sup>25</sup> Relapse of the initial tuberculosis infection with acquired resistance to rifampin is the best explanation of the patient's second illness.

Our patient received six months of directly observed antituberculosis therapy, the regimen recommended by the American Thoracic Society and the Centers for Disease Control and Prevention (CDC).<sup>26</sup> Although some authorities, including the CDC Advisory Committee for the Elimination of Tuberculosis, have recommended nine months of treatment for HIV-associated tuberculosis,<sup>27</sup> six-month regimens have resulted in very low rates of relapse.<sup>28,29</sup> Moreover, our patient took the prescribed antituberculosis drugs under direct observation, an approach that reduces the risk of relapse and the emergence of drug-resistant disease, even in patients with HIV infection.<sup>30,31</sup> Nonetheless, a small percentage of patients with adequately treated tuberculosis will relapse, and the proportion of HIV-infected patients who relapse may be decreased by prolonging therapy.<sup>32</sup> Our patient was severely immunocompromised at the time of his initial therapy. In these circumstances, the probability of relapse is likely to be increased. This is the only relapse among the more than 80 HIV-infected patients with tuberculosis we have treated with a six-month supervised regimen (unpublished data).

It is not clear whether the strain of tuberculosis became resistant while the patient was taking rifabutin or

whether it was already resistant before prophylaxis with rifabutin was begun. Sequencing of the *rpoB* gene of the two *M. tuberculosis* isolates revealed a point mutation resulting in the substitution of a single amino acid at codon 531. The substitution of leucine for serine at this position has been noted in a large proportion of rifampin-resistant strains of *M. tuberculosis*.<sup>20</sup> This mutation is also associated with high-level resistance to rifabutin in vitro.<sup>33</sup> Relapse of rifampin-resistant tuberculosis with substitution of a single amino acid in the *rpoB* gene product has been reported by Nolan and coworkers in three HIV-infected patients who were not treated with rifabutin but who had received rifampin.<sup>34</sup> They postulated that in addition to poor compliance with therapy, malabsorption of isoniazid or altered drug metabolism could have contributed to the appearance of rifampin resistance. Our patient was compliant with therapy and had no apparent gastrointestinal or hepatic disease that would alter drug absorption or metabolism.

Weltman and colleagues have reported a case of primary rifampin-resistant tuberculosis in a prison inmate taking rifabutin as prophylaxis against *M. avium* complex infection.<sup>12</sup> In that case, exposure to an infectious inoculum of *M. tuberculosis* that was susceptible to rifampin while the patient was taking rifabutin apparently resulted in the emergence of resistance to rifampin and rifabutin. Although our patient's compliance with rifampin therapy was closely monitored, it is possible that he took rifabutin irregularly. If he began taking rifabutin only after the occurrence of symptoms, it is very likely that this would have selected for rifampin-resistant mutants. Alternatively, rifampin resistance may have been present from the outset of the relapse, and rifabutin may therefore have been ineffective in suppressing disease.

In patients with active tuberculosis, multidrug therapy is necessary to prevent the emergence of drug resistance because of the presence of innately resistant mutants in populations of replicating *M. tuberculosis*. The prevalence of rifampin-resistant mutants is approximately 1 in 10<sup>8</sup> to 1 in 10<sup>10</sup> bacteria.<sup>35</sup> Exposure to rifampin or rifabutin alone when the number of tuberculosis organisms exceeds this level would select for resistant mutants. Conversely, the use of these agents when the mycobacterial burden is less than 10<sup>8</sup> should effectively suppress bacterial replication. In fact, monotherapy with either rifampin or rifabutin is effective in animal models of chronic tuberculosis,<sup>36,37</sup> and rifampin prophylaxis has been efficacious in men with silicosis.<sup>38</sup> Thus, the emergence of rifampin-resistant tuberculosis during preventive therapy with rifabutin suggests either the presence of a heavy preexisting *M. tuberculosis* infection or failure to achieve consistently adequate levels of rifabutin in the blood.

Despite physicians' fears, few cases of rifampin-resistant tuberculosis have been reported in patients taking rifabutin,<sup>12</sup> and it is possible that rifabutin protects against tuberculosis as well as *M. avium* complex

infection. Clarithromycin and azithromycin are possible alternatives to rifabutin as prophylaxis against *M. avium* complex infection that would not increase the risk of drug-resistant tuberculosis.<sup>39,40</sup> Guidelines issued by the Public Health Service and the Infectious Disease Society of America<sup>41</sup> recommend screening all patients for active tuberculosis and obtaining mycobacterial blood cultures before rifabutin prophylaxis is begun. Our patient did not undergo any such screening before his physician prescribed rifabutin. Adherence to these guidelines might have detected the relapse of tuberculosis and averted the initiation of rifabutin therapy.<sup>42</sup> Careful follow-up of HIV-infected patients with treated tuberculosis is essential, and physicians should follow the published guidelines<sup>41</sup> for the use of rifabutin as prophylaxis against *M. avium* complex disease.

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## REFERENCES

- Bloch AB, Cauthen GM, Onorato IM, et al. Nationwide survey of drug-resistant tuberculosis in the United States. *JAMA* 1994;271:665-71.
- Nosocomial transmission of multidrug-resistant tuberculosis among HIV-infected persons — Florida and New York, 1988–1991. *MMWR Morb Mortal Wkly Rep* 1991;40:585-91.
- Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen GM, Dooley SW. The emergence of drug-resistant tuberculosis in New York City. *N Engl J Med* 1993;328:521-6. [Erratum, *N Engl J Med* 1993;329:148.]
- Iseman MD. Treatment of multidrug-resistant tuberculosis. *N Engl J Med* 1993;329:784-91.
- Goble M, Iseman MD, Madsen LA, Waite D, Ackerson L, Horsburgh CR Jr. Treatment of 171 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. *N Engl J Med* 1993;328:527-32.
- Telzak EE, Sepkowitz K, Alpert P, et al. Multidrug-resistant tuberculosis in patients without HIV infection. *N Engl J Med* 1995;333:907-11.
- Turett GS, Telzak EE, Torian LV, et al. Improved outcomes for patients with multidrug-resistant tuberculosis. *Clin Infect Dis* 1995;21:1238-44.
- Salomon N, Perlman DC, Friedmann P, Buchstein S, Kreiswirth BN, Milman D. Predictors and outcome of multidrug-resistant tuberculosis. *Clin Infect Dis* 1995;21:1245-52.
- Nightingale SD, Cameron DW, Gordin FM, et al. Two controlled trials of rifabutin prophylaxis against *Mycobacterium avium* complex infection in AIDS. *N Engl J Med* 1993;329:828-33.
- Public Health Service Task Force on Prophylaxis and Therapy for *Mycobacterium avium* Complex. Recommendations on prophylaxis and therapy for disseminated *Mycobacterium avium* complex for adults and adolescents infected with human immunodeficiency virus. *MMWR Morb Mortal Wkly Rep* 1993;42(RR-9):14-20.
- Reichman LB, McDonald RJ, Mangura BT. Rifabutin prophylaxis against *Mycobacterium avium* complex infection. *N Engl J Med* 1994;330:437-8.
- Weltman AC, Righi SP, DiFerdinando GT Jr, Jovell RJ, Driscoll JR. Rifampicin-resistant *Mycobacterium tuberculosis*. *Lancet* 1995;345:1513.
- Chaulk CP, Moore-Rice K, Rizzo R, Chaisson RE. Eleven years of community-based directly observed therapy for tuberculosis. *JAMA* 1995;274:945-51.
- Siddiqi SH. Radiometric (BACTEC) tests for slowly growing mycobacteria. In: Isenberg HD, ed. *Clinical microbiology procedures handbook*. Vol. 1. Washington, D.C.: American Society for Microbiology, 1992:5.14.1-5.14.25.
- Kent PT, Kubica GP. *Public health mycobacteriology: a guide for the level III laboratory*. Atlanta: Department of Health and Human Services, 1985.
- Cave MD, Eisenach KD, McDermott PF, Bates JH, Crawford JT. IS6110: conservation of sequence in the *Mycobacterium tuberculosis* complex and its utilization in DNA fingerprinting. *Mol Cell Probes* 1991;5:73-80.
- van Embden JA, Cave MD, Crawford JT, et al. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. *J Clin Microbiol* 1993;31:406-9.
- Ross BC, Raios K, Jackson K, Dwyer B. Molecular cloning of a highly repeated DNA element from *Mycobacterium tuberculosis* and its use as an epidemiological tool. *J Clin Microbiol* 1992;30:942-6.

19. Poulet S, Cole ST. Characterization of the highly abundant polymorphic GC-rich-repetitive sequence (PGRS) present in *Mycobacterium tuberculosis*. Arch Microbiol 1995;163:87-95.
20. Telenti A, Imboden P, Marchesi F, et al. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. Lancet 1993;341:647-50.
21. Jin DJ, Gross CA. Mapping and sequencing of mutations in the *Escherichia coli rpoB* gene that lead to rifampicin resistance. J Mol Biol 1988;202:45-58.
22. Honore N, Cole ST. Molecular basis of rifampin resistance in *Mycobacterium leprae*. Antimicrob Agents Chemother 1993;37:414-8.
23. Miller LP, Crawford JT, Shinnick TM. The *rpoB* gene of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1994;38:805-11.
24. Graham NMH, Harrington S, Libonati JP, et al. Molecular epidemiology of TB in Baltimore City: preliminary data. Am J Respir Crit Care Med 1995; 151:Suppl:A145. abstract.
25. Small PM, Shafer RW, Hopewell PC, et al. Exogenous reinfection with multidrug-resistant *Mycobacterium tuberculosis* in patients with advanced HIV infection. N Engl J Med 1993;328:1137-44.
26. Bass JB Jr, Farer LS, Hopewell PC, et al. Treatment of tuberculosis and tuberculosis infection in adults and children. Am J Respir Crit Care Med 1994;149:1359-74.
27. Tuberculosis and human immunodeficiency virus infection: recommendations of the Advisory Committee for the Elimination of Tuberculosis (ACET). MMWR Morb Mortal Wkly Rep 1989;38:236-50.
28. Small PM, Schecter GF, Goodman PC, Sande MA, Chaisson RE, Hopewell PC. Treatment of tuberculosis in patients with advanced human immunodeficiency virus infection. N Engl J Med 1991;324:289-94.
29. Chaisson RE, Clermont HC, Holt EA, et al. Six-month supervised intermittent tuberculosis therapy in Haitian patients with and without HIV infection. Am J Respir Crit Care Med (in press).
30. Weis SE, Slocom PC, Blais FX, et al. The effect of directly observed therapy on the rates of drug resistance and relapse in tuberculosis. N Engl J Med 1994;330:1179-84.
31. Alwood K, Keruly JC, Moore-Rice K, Stanton DL, Chaulk CP, Chaisson RE. Effectiveness of supervised, intermittent therapy for tuberculosis in HIV-infected patients. AIDS 1994;8:1103-8.
32. Perriens JH, St Louis ME, Mukadi YB, et al. Pulmonary tuberculosis in HIV-infected patients in Zaire: a controlled trial of treatment for either 6 or 12 months. N Engl J Med 1995;332:779-84.
33. Bodmer T, Zurcher G, Imboden P, Telenti A. Mutation position and type of substitution in the  $\beta$ -subunit of RNA polymerase influence in-vitro activity of rifamycins in rifampicin-resistant *Mycobacterium tuberculosis*. J Antimicrob Chemother 1995;35:345-8.
34. Nolan CM, Williams DL, Cave MD, et al. Evolution of rifampin resistance in human immunodeficiency virus-associated tuberculosis. Am J Respir Crit Care Med 1995;152:1067-71.
35. Cole ST. *Mycobacterium tuberculosis*: drug-resistance mechanisms. Trends Microbiol 1994;2:411-5.
36. Ji B, Truffot-Pernot C, Lacroix C, et al. Effectiveness of rifampin, rifabutin, and rifapentine for preventive therapy of tuberculosis in mice. Am Rev Respir Dis 1993;148:1541-6.
37. Jabes D, Della Bruna C, Rossi R, Oliario P. Effectiveness of rifabutin alone or in combination with isoniazid in preventive therapy of mouse tuberculosis. Antimicrob Agents Chemother 1994;38:2346-50.
38. Hong Kong Chest Service/Tuberculosis Research Council, Madras/British Medical Research Council. A double-blind placebo-controlled clinical trial of three antituberculosis chemoprophylaxis regimens in patients with silicosis in Hong Kong. Am Rev Respir Dis 1992;145:36-41.
39. Pierce M, Crampton S, Henry D, Craft C, Notario G. The effect of MAC and its prevention on survival in patients with advanced HIV infection. In: Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, September 17-20, 1995. Washington, D.C.: American Society for Microbiology, 1995. abstract.
40. Havlir DV, McCutchan JA, Bozzette SA, Dunn M. A double-blind, randomized study of weekly azithromycin, daily rifabutin, and combination azithromycin and rifabutin for the prevention of *Mycobacterium avium* complex (MAC) in AIDS patients. In: Abstracts of the Third Conference on Retroviruses and Opportunistic Infections, Washington, D.C., January 28-February 2, 1996. abstract (in press).
41. USPHS/IDSA guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus: a summary. MMWR Morb Mortal Wkly Rep 1995;44(RR-8):1-34.
42. Jones BE, Young SMM, Antoniskis D, Davidson PT, Kramer F, Barnes PF. Relationship of the manifestations of tuberculosis to CD4 cell counts in patients with human immunodeficiency virus infection. Am Rev Respir Dis 1993;148:1292-7.

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