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AN OUTBREAK INVOLVING EXTENSIVE TRANSMISSION OF A VIRULENT STRAIN OF *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Background and Methods From 1994 to 1996, there was a large outbreak of tuberculosis in a small, rural community with a population at low risk for tuberculosis. Twenty-one patients with tuberculosis (15 with positive cultures) were identified; the DNA fingerprints of the 13 isolates available for testing were identical. To determine the extent of transmission, we investigated both the close and casual contacts of the patients. Using a mouse model, we also studied the virulence of the strain of *Mycobacterium tuberculosis* that caused the outbreak.

Results The index patient, in whom tuberculosis was diagnosed in 1995; the source patient, in whom the disease was diagnosed in 1994; and a patient in whom the disease was diagnosed in 1996 infected the other 18 persons. In five, active disease developed after only brief, casual exposure. There was extensive transmission from the three patients to both close and casual contacts. Of the 429 contacts, 311 (72 percent) had positive skin tests, including 86 with documented skin-test conversions. Mice infected with the virulent Erdman strain of *M. tuberculosis* had approximately 1000 bacilli per lung after 10 days and about 10,000 bacilli per lung after 20 days. In contrast, mice infected with the strain involved in the outbreak had about 10,000 bacilli per lung after 10 days and about 10 million bacilli per lung after 20 days.

Conclusions In this outbreak of tuberculosis, the growth characteristics of the strain involved greatly exceeded those of other clinical isolates of *M. tuberculosis*. The extensive transmission of tuberculosis may have been due to the increased virulence of the strain rather than to environmental factors or patient characteristics. (N Engl J Med 1998;338:633-9.)

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OVER the past few decades, numerous outbreaks of tuberculosis have been reported in hospitals, prisons, schools, homeless shelters, bars, and factories. In some outbreaks the transmission of *Mycobacterium tuberculosis* was limited, whereas in others, there were high rates of transmission.¹⁻¹⁰ Transmission has also been reported after minimal exposure to an infectious patient.^{4,5,10-13} The variability in transmission rates has been attributed to the environment in which the outbreak occurred and to the clinical characteristics of the source patient. Some investigators have also postulated that the strains involved in such outbreaks may be especially virulent.^{6,9,12} Although the virulence of various *M. tuberculosis* isolates has been studied in animal models,^{14,15} it has not been examined in relation to transmission during a specific outbreak.

In May 1995, investigation of a preschool child with a positive tuberculin test identified an uncle as the index patient. The uncle lived in a rural area and worked in a clothing factory in the neighboring county. The two counties in Tennessee and Kentucky involved in this investigation had a total population of approximately 14,000, and each county averaged less than one case of tuberculosis per year from 1985 through 1993. We report the results of

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our investigation, which documented extensive transmission of *M. tuberculosis* from the index patient, the source patient, and a secondary source to residents of this area with a previously low incidence. Because of the very high rates of skin-test positivity and strong immune response to the purified protein derivative (PPD) among contacts, we studied the virulence of the strain of *M. tuberculosis* involved in the outbreak, which was designated CDC (Centers for Disease Control and Prevention) 1551, as well as CSU 93 as part of the project to characterize the virulence of *M. tuberculosis* sponsored by the National Institutes of Health.

METHODS

Index Patient

The index patient was a 21-year-old white man, born in the United States and negative for human immunodeficiency virus, who lived in a rural area and had worked in a clothing factory in a nearby state since the fall of 1994. About one month after beginning work, he presented with chest pain and cough and was given a diagnosis of pneumonia, for which unspecified antibiotics were prescribed. He reported improvement but contacted his physician two months later because of recurrent cough, for which he was given erythromycin. In early 1995, cough medicine was prescribed for his continuing cough. When his niece was found to have a positive tuberculin test in April 1995, the family was screened and he was given a diagnosis of cavitary tuberculosis. He had no symptoms suggestive of laryngeal tuberculosis. Smears of sputum specimens contained numerous acid-fast bacilli, and sputum cultures were positive for *M. tuberculosis*. The isolates were susceptible to antituberculosis medications.

Identification of Patients with Tuberculosis

To identify patients with tuberculosis, we cross-matched the index patient's list of social and work contacts with state tuberculosis registries from 1994 through 1996. We reviewed the records of the state mycobacteriology laboratories to identify persons from the area with positive *M. tuberculosis* cultures, and we examined pharmacy logs from county health departments to identify persons taking antituberculosis medications. The medical records for these patients were reviewed, and the patients with tuberculosis were interviewed.

Tuberculin-Test Screening

The immediate and extended family of the index patient, his coworkers, and close as well as casual social contacts underwent tuberculin-test screening. Screening, conducted in late May and early June 1995 with follow-up testing in the fall, was done with the Mantoux method with 5 TU of tuberculin PPD (Tubersol, Connaught Laboratories, Swiftwater, Pa.). Persons with a history of a positive test or evidence of prior tuberculosis were not retested. For epidemiologic analyses, we defined a positive test as one in which there was 10 mm or more of induration, and a conversion in the result from negative to positive as the finding of an increase in induration of 10 mm or more in the previous two years.

To check for errors in performing tuberculin tests, we analyzed results according to the nurse who administered the test and the nurse who read the test. Because of concern that the lots of PPD used may have had impurities or other problems that could have contributed to the unusually large reactions seen, we contacted neighboring counties and the Food and Drug Administration (FDA) to determine whether problems had been noted with the lots of PPD used in this investigation.

Identification of the Source Patient

In an effort to identify the source of the infection, we interviewed the index patient and retrospectively reviewed the charts of all patients in the area who had been given a diagnosis of tuberculosis since 1990. Members of the local health departments were interviewed to identify potential connections between any of these patients and the index patient. Any patients with potential links to the index patient were interviewed.

Laboratory and Virulence Studies

Strains of *M. tuberculosis* isolated from patients in this outbreak and from other patients with tuberculosis in the 10 surrounding counties were sent to the CDC for DNA-fingerprint analysis with the IS6110 insertion sequence.¹⁶ Secondary typing of isolates involved in the outbreak was performed with pTBN12 probes.¹⁷ To investigate the virulence of the organism involved in the outbreak, its growth in the lungs of infected C57BL/6 mice was compared with that of the virulent laboratory strain *M. tuberculosis* Erdman (which is passed through mice to avoid the loss of virulence) with conventional procedures.¹³ For these studies, passage of the clinical strains on laboratory medium was kept to a minimum to avoid changes in virulence. Bacilli were grown to the mid-log phase in Proskauer Beck medium, and the number of viable bacilli per milliliter was determined by plating portions of the culture on nutrient 7H11 agar. The cultures were stored at -70°C while viability was determined.

For the in vivo studies, frozen samples were thawed and diluted in sterile pyrogen-free saline to a concentration of 50,000 viable bacilli per milliliter. Then, 10 ml was added to the Venturi nebulizer unit of a Middlebrook Aerosol Generation device (Glas-Col, Terre Haute, Ind.), and C57BL/6 mice were exposed to an aerosol for 30 minutes, which typically results in the implantation of about 100 bacilli in the lungs of the mice. We monitored the numbers of bacilli in the mouse lungs by using carbon dioxide inhalation to kill four mice per time point for each isolate, plating serial dilutions of individual whole-organ homogenates on nutrient 7H11 agar, and counting the colonies after two to three weeks of incubation at 37°C in humidified air.

RESULTS

Identification of Patients with Tuberculosis

From May 1995 through November 1995, five secondary cases of tuberculosis were identified among family members and coworkers of the index patient. One relative, who was also a coworker, had smear-negative, culture-positive pulmonary tuberculosis. Another family member had abnormal findings on a chest radiograph that resolved with therapy and was given a diagnosis of smear-negative and culture-negative pulmonary tuberculosis. Pleural tuberculosis was diagnosed in one coworker; no specimens were obtained. Tuberculosis was also diagnosed in two relatives who lived more than 60 miles away and whose only exposures to the index patient were for two to four hours on Christmas and Easter. One of these relatives underwent surgery for symptoms thought to be related to rheumatoid arthritis and had a culture-positive specimen from synovial fluid from a finger joint. This patient had been receiving long-term therapy with steroids for arthritis and had normal findings on chest radiographs. The other relative was given a diagnosis of extrapulmonary tuberculosis; a chest radiograph showed hilar adenopathy, which improved with therapy.

Tuberculin-Test Screening

A total of 338 contacts of the index patient were identified. All were non-Hispanic whites, and all but one had been born in the United States. Contacts included family members, close friends, and casual social acquaintances. Among the casual contacts, the most frequently reported activity that brought them into contact with the index patient was "hanging out" at the local gasoline station at night, mostly outside in the open air.

The results of screening were obtained for 328 contacts (97 percent): 224 (68 percent) had positive tests (≥ 10 mm of induration), and 6 others had induration of 7 to 9 mm (Table 1). Fifty-one persons had documented skin-test conversions: 4 close contacts (2 of whom were also coworkers), 13 casual social contacts, and 34 coworkers. Thirty-six conversions occurred between spring 1995 and follow-up testing in the fall; the other 15 had documented negative skin tests (all 0 mm of induration) 9 to 24 months before their positive test in the spring of 1995. As compared with 149 community members with no identified contact with the index patient who requested testing after hearing about the outbreak, all contacts of the index patient had a significantly higher risk of a positive skin test; relative risks ranged from 17.0 for casual social contacts to 37.3 for close contacts (Table 1).

Among the 224 contacts with positive skin tests, only 8 had other potential risk factors for a positive test. One foreign-born contact had received bacille Calmette-Guérin vaccine, and seven may have been exposed in the past to a family member with tuberculosis; most of these potential exposures had occurred more than 10 years earlier. All infected contacts were prescribed a six-month course of isoniazid as preventive therapy.

The millimeters of induration were available for 212 (95 percent) of the contacts with positive skin tests: 68 (32 percent) had induration of 20 mm or more, and at least 25 had vesiculated lesions. There was no association between the presence of vesiculated lesions and the PPD lot used or the nurse who administered or read the skin test. The PPD lots used in this investigation were also used to screen persons with no contact with the index patient, in other contact investigations in more than 10 counties in the area, and for routine screening activities (e.g., among schoolchildren). In no case had high rates of positive reactions, abnormally large reactions, or any vesiculated lesions been reported. The FDA had received no reports of adverse events or unusual reactions in association with these PPD lot numbers.

Workplace of the Index Patient

The factory where the index patient worked had one large open work area approximately 61 m by 43 m (200 ft by 140 ft) with a ceiling height of

TABLE 1. RESULTS OF TUBERCULIN-TEST SCREENING AMONG 328 CONTACTS OF THE INDEX PATIENT AND 149 COMMUNITY MEMBERS WITH NO APPARENT CONTACT WITH THE INDEX PATIENT.

TYPE OF CONTACT*	NO. POSITIVE/ NO. TESTED (%)	RELATIVE RISK OF A POSITIVE TEST (95% CI)†
Close	28/28 (100)	37.3 (14.2–98.0)
Coworker only	165/232 (71)	26.5 (10.0–69.9)
Casual social	31/68 (46)	17.0 (6.2–46.2)
None	4/149 (3)	1.0‡

*All categories are mutually exclusive. Close contacts consisted of immediate family members, close friends, and 13 coworkers who were also friends. Casual social acquaintances had only occasional or sporadic contact with the index patient, such as while "hanging out" outside the local gasoline station at night. In addition, 149 worried community members with no apparent contact with the index patient asked to be tested after hearing about the outbreak.

†CI denotes confidence interval.

‡This is the reference group.

more than 6 m (20 ft). There were approximately 250 work stations about 1.5 to 2 m (5 to 6 ft) apart. Among coworkers, no statistically significant differences between those with positive skin tests and those with negative tests were found with regard to age, sex, county of residence, lunch shift, or work assignment. None of the 12 coworkers who stopped work before March 1995 had positive skin tests. Analyses of air-flow movement and tracer-gas evaluations with standard techniques¹⁸ showed that there was excellent movement of air throughout the factory, with approximately a 0.4 air exchange with outside air per hour.

Identification of the Source Patient

Retrospective investigation identified a patient given a diagnosis of tuberculosis in 1994 as the most likely source. The index patient was not identified as a contact of this patient in 1994. In 1995, however, the index patient reported infrequent contact with the source patient shortly before tuberculosis was diagnosed in the latter. The diagnosis in the source patient, which was based on the findings of smear-positive (numerous acid-fast bacilli) and culture-positive cavitary disease, was made in July 1994, after the investigation of a young child with a positive skin test in his family. The source patient had reported hoarseness and was seen by an otolaryngologist in the months before his diagnosis. Indirect laryngoscopy demonstrated some leukoplakia and small polyps on each side of the larynx that were attributed to chronic smoking. No biopsy specimens were obtained, and no changes were seen in the patient's larynx over a period of two months. When inter-

viewed in 1995, the otolaryngologist stated that the source patient's clinical picture was not consistent with a diagnosis of laryngeal tuberculosis. None of the staff members in this physician's office had positive tuberculosis tests when screened in 1994 and 1995. The 1994 investigation of the source patient identified four secondary cases: three in family members and one in a family friend. Extensive transmission among the contacts of this patient was also documented: 37 of 42 contacts (88 percent) had positive skin tests, and 8 of the 37 had documented skin-test conversions.

Additional Cases

From January through December 1996, 10 additional cases associated with the outbreak were diagnosed, all in the county in which the index patient and the source patient resided. Seven cases were culture-positive; the other three had epidemiologic links to cases associated with the outbreak and clinical and radiographic evidence of tuberculosis (positive skin tests and either hilar adenopathy or pleural effusion). Five cases were in contacts of either the index patient or the source patient who were not identified during contact investigations of those patients. In the case of two other patients identified, contact with either the index patient or the source patient appeared to have been casual and sporadic, and in the case of one, no contact with anyone with tuberculosis associated with the outbreak was identified. The only exposure in the case of the remaining two patients was in a physician's office one afternoon in 1996, when another patient in whom tuberculosis was diagnosed approximately two months later was also in the waiting area (the secondary source). Tuberculosis was diagnosed in these two patients — a two-year-old with extensive culture-positive pulmonary disease and an adult — nine months after this exposure. The investigation of the secondary source patient who apparently infected them showed that the rate of transmission was similar to that for the index patient and the source patient: of 59 contacts, 50 (85 percent) had positive tests, and 7 of the 50 had vesiculated lesions. Skin-test conversions were documented in 22 contacts, including 8 of the 16 staff members of the physician's office (50 percent).

Laboratory and Virulence Studies

Thirty-eight isolates of *M. tuberculosis* were sent to the CDC for DNA-fingerprint analyses with IS6110: isolates from 13 of the 15 culture-positive cases in the outbreak and isolates from 25 cases in surrounding counties that were not associated with the outbreak. All 13 isolates associated with the outbreak were susceptible to antituberculosis medication, and all had the same four-banded DNA fingerprint (Fig. 1). Secondary typing with pTBN12 was performed on 8 of these 13 isolates; all had the same finger-

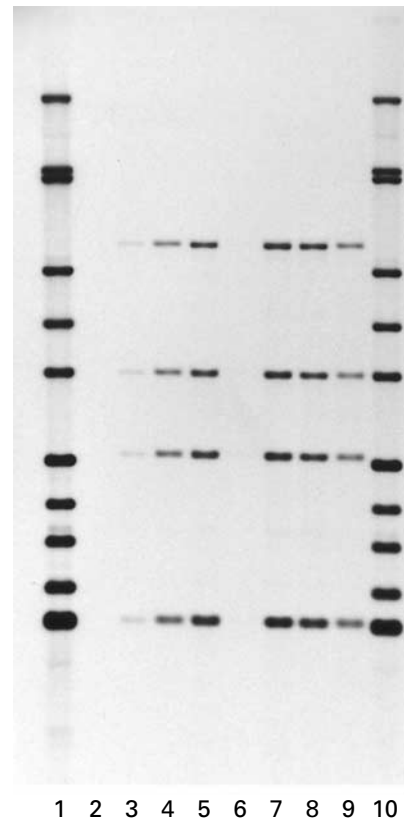


Figure 1. IS6110 Subtyping of *M. tuberculosis* Isolates.

Isolates from the outbreak (lanes 3, 4, 5, 7, 8, and 9) were subtyped with the use of IS6110 restriction-fragment-length polymorphisms as previously described.¹⁶ Results for the reference strain, MT14323, are shown in lanes 1 and 10. Lane 2 is empty. In lane 6 the sample contained insufficient DNA.

print (Fig. 2). The remaining 25 isolates had 23 different IS6110-based DNA fingerprints, none of which matched that of the strain involved in the outbreak.

As is typically observed in mice infected with approximately 100 colony-forming units of *M. tuberculosis* by means of an aerosol, growth of the virulent laboratory strain Erdman was logarithmic, with approximately 1000 bacilli per lung after 10 days and approximately 10,000 bacilli per lung after 20 days. In contrast, mice infected with the isolate from the index patient had approximately 10,000 bacilli per lung after 10 days and 10 million bacilli per lung after 20 days (Fig. 3). The growth of the isolate from the source patient was also accelerated, though it was somewhat less than that of the isolate from the index patient. This extraordinary growth (both the rate and the extent) greatly exceeds that seen with other clinical isolates of *M. tuberculosis*, whose growth usually falls in the range of ± 1.5 logs of that of the Erdman strain.¹⁴ For example, the highly virulent

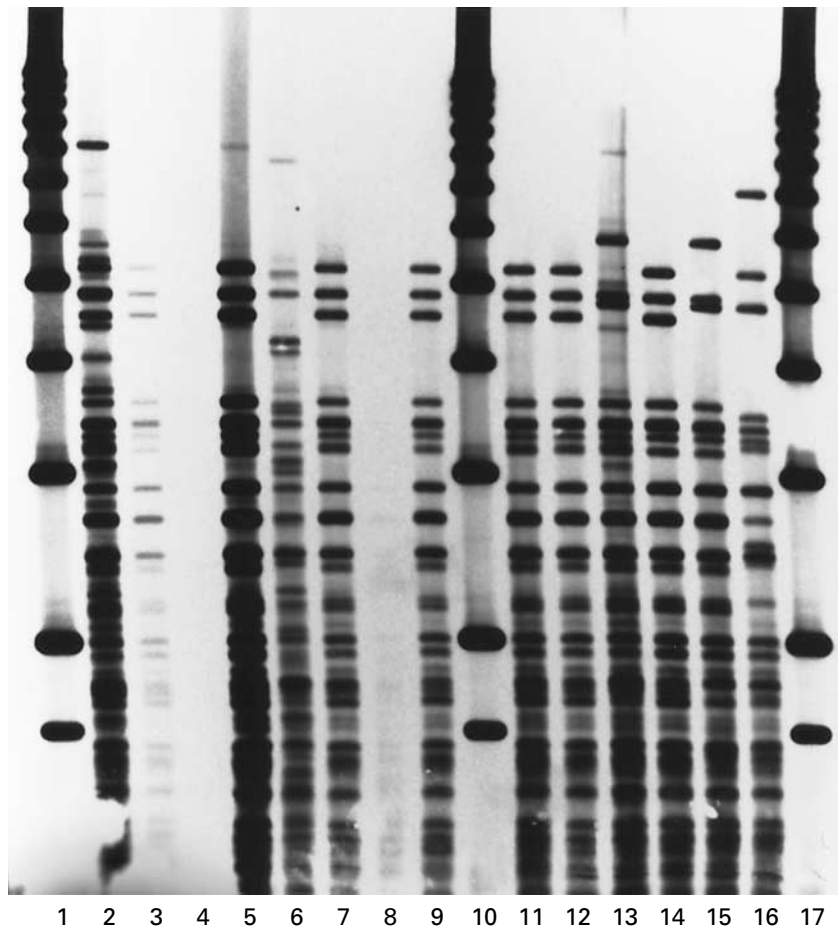


Figure 2. pTBN12 Subtyping of *M. tuberculosis* Isolates. Isolates from the outbreak (lanes 3, 5, 7, 8, 9, 11, 12, and 14) were subtyped with the use of pTBN12 restriction-fragment-length polymorphisms as previously described.¹⁷ Molecular-weight markers are shown in lanes 1, 10, and 17, and lanes 2, 6, 13, 15, and 16 show isolates unrelated to the outbreak. Lane 4 is empty.

strain CSU 19, a so-called fast-growing strain of tuberculosis, only reaches about 300,000 bacilli per lung after 20 days' growth (which is about 30 times that of the Erdman strain but about 1/33 that of the strain involved in the outbreak).¹⁴ The growth of the strain involved in the outbreak was also measured in the mouse spleen. On day 20, the growth of the isolate from the index patient was about 70 times that of the Erdman strain and the growth of the isolate from the source patient was about 60 times that of the Erdman strain.

DISCUSSION

Our investigation documents the extensive transmission of *M. tuberculosis* in a rural population with minimal risk factors for tuberculosis. From 1994 through 1996, 21 cases related to the outbreak were identified. The delay in diagnosis and the extent of

disease in the index patient could help explain the extensive transmission among his contacts. However, the pattern of transmission to his coworkers suggests that the index patient was not infectious until early or mid-March 1995, or 10 to 12 weeks before diagnosis. Although the excellent movement of air throughout the factory where he worked and the low rate of air exchange in the factory may have contributed to transmission in that facility, this does not explain why the infection was transmitted to so many contacts who had extremely limited exposure to the index patient, primarily in an outdoor setting, or to secondary case patients, who became infected and in whom tuberculosis developed after exposure of only two to four hours. In addition, extensive transmission was also seen among close and casual contacts of the source patient in 1994 and the secondary source patient in 1996. In particular, active

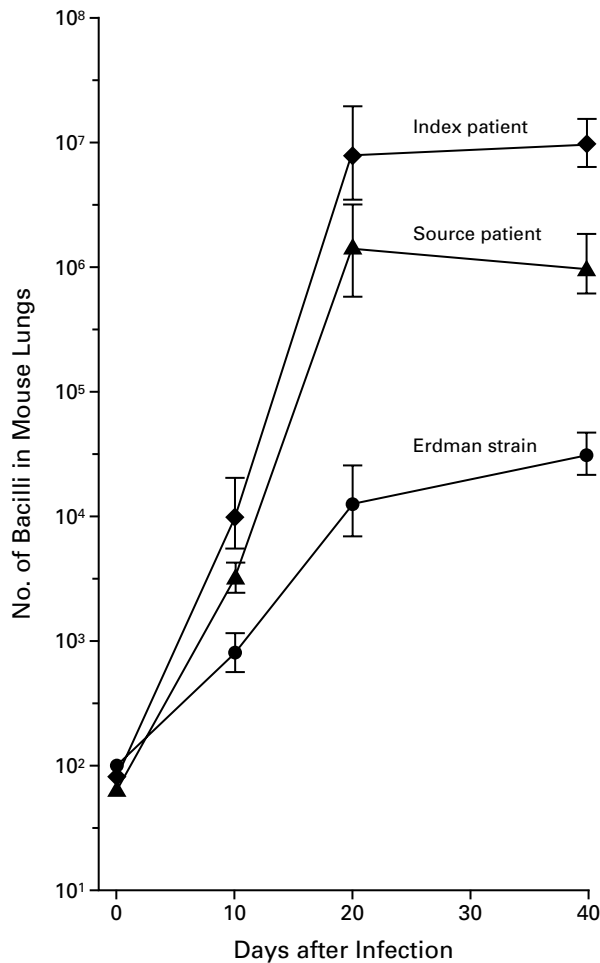


Figure 3. Growth of *M. tuberculosis* Isolates in Vivo. C57BL/6 mice were infected with the isolate from the index patient, the isolate from the source patient, or the virulent laboratory standard *M. tuberculosis* Erdman by the aerosol route, and the number of bacilli in the lungs was measured 10 days, 20 days, and 40 days after infection. The 1 bars indicate standard errors.

disease was documented in two patients who had very limited exposure to the latter patient — a short period in a physician's clinic on one afternoon. These data suggest that rather than being due to environmental factors or patient characteristics, the increased rate of transmission was primarily a feature of the strain of *M. tuberculosis* involved, such as increased virulence or possibly an increased ability to survive in aerosol.

As a first step in our investigation of virulence, we measured the ability of the strain involved in the outbreak to grow in the murine model of tuberculosis. This animal model is used to assess the overall ability of a strain to enter the lung, infect alveolar macrophages, and survive and replicate within mac-

rophages. The results suggest that the initial implantation and infection steps were similar for the strain involved in the outbreak and other virulent strains, because similar numbers of bacilli from these strains were found in the lungs shortly after the standardized aerosol exposure. Subsequently, the outbreak strain displayed a rate and extent of growth that greatly exceeded those of virulent laboratory strains and recent clinical isolates of *M. tuberculosis*, which grow at virtually the same rate and to the same extent as the standard laboratory strain Erdman.¹⁴

The strain involved in the outbreak grew much better in vivo than commonly encountered strains, and this difference could be a factor in both the high rate of transmission and the strong immunoreactivity to PPD of this strain. A faster rate of growth might increase the likelihood that bacilli could establish a focus of infection before being eliminated by the immune response. In addition, the faster growth rate should increase the amounts of mycobacterial antigens being presented to the immune system during the induction of the cellular immune response to the tubercle bacillus. Thus, the unusually vigorous reactions to PPD could reflect larger-than-usual immunizing doses of mycobacterial antigens. This possibility is supported by preliminary results of studies with human monocytes suggesting that the strain involved in the outbreak grows slightly more rapidly intracellularly (mean [\pm SE] generation time, 25 ± 4 hours) than the virulent laboratory strain H37Rv (generation time, 31 ± 3 hours) and induces larger amounts of cytokines, including about twice as much tumor necrosis factor α (Kaplan G, Manca C, Rockefeller University: personal communication). The increased intracellular growth rate and increased production of tumor necrosis factor α are consistent with the finding of the greater rapidity and extent of growth of this strain in mice, because both factors are associated with increased growth in vivo.¹⁹ The increased cytokine production is also consistent with the finding of unusually vigorous tuberculin-test responses.

Infection with this strain was not associated with increased rates of active tuberculosis, perhaps because of the effect of the investigation of the index patient and of tuberculosis-control efforts. For example, the infectious patients may have been infectious for only a short period, and aggressive investigation of contacts was begun as soon as an outbreak was suspected in 1995. As a result, infected persons began receiving isoniazid prophylactically relatively soon after being infected. Thus, the number of secondary cases in this outbreak was small as compared with the number of infections, possibly reflecting the value of a contact investigation and prophylaxis with isoniazid.

The strain involved in this outbreak has been selected for the *M. tuberculosis* genome-sequencing

project.²⁰ Further analysis of the extraordinary ability of this strain to grow in an animal model may help elucidate details of the transmission and pathogenicity of *M. tuberculosis*. Studies to identify the genes and gene products involved in these processes could lead to the identification of virulence factors of the tubercle bacillus that are important for growth in vivo or the development or transmission of disease. This, in turn, could lead to the development of a subunit vaccine that targets a critical virulence factor or a test to identify persons at high risk for active disease. Similarly, the study of the antigens responsible for eliciting the vigorous PPD responses may identify antigens that could be used as skin-test reagents to identify persons infected with a highly virulent strain or persons in whom the bacilli are actively replicating. Identification of persons at high risk for active tuberculosis would allow us to direct tuberculosis-control efforts to those most in need of preventive therapy and most likely to become a source for the further spread of the disease.

There probably remains a large reservoir of persons in the area in which this outbreak occurred who are infected with *M. tuberculosis*, and active disease is likely to develop in some. Because of the unusual transmission characteristics of this strain of *M. tuberculosis*, it will be important to maintain active surveillance in this area.

Preliminary results of this investigation were presented at the American Thoracic Society Meeting, New Orleans, May 11-15, 1996 (abstract A334), and the Infectious Disease Society of America Meeting, San Francisco, September 14-17, 1997 (abstract 35).

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