Nosocomial Transmission of *Mycobacterium bovis* Bacille Calmette-Guerin to Children Receiving Cancer Therapy and to Their Health Care Providers

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A previous report of nosocomial infection due to *Mycobacterium bovis* bacille Calmette-Guerin (BCG) implicated contamination of chemotherapy solutions reconstituted under the same biosafety hood as BCG vaccine used for bladder cancer therapy. We report 3 similar BCG infections in children and describe evidence of respiratory transmission to health care workers (HCWs) from 1 patient. These children were receiving chemotherapy for leukemia when they presented with active tuberculosis. Each isolate was identified biochemically and by both gas-liquid chromatography and major polymorphic tandem repeat–polymerase chain reaction. Pulsed-field gel electrophoresis showed that 2 isolates were identical strains and identical to the Tice and Connaught strains licensed in the United States for bladder chemotherapy. The third isolate differed by a single fragment after Dral restriction. One patient with heavily positive sputum exposed numerous HCWs. Of 41 HCWs, 2 (5%) converted their purified protein derivatives (PPD) skin test. These data underscore the risk of nosocomial BCG transmission by contamination of chemotherapy solutions and demonstrate the potential for transmission to HCWs from patients with active pulmonary disease.

Bacille Calmette-Guerin (BCG), a live attenuated bacterial vaccine derived from *Mycobacterium bovis*, is used in many parts of the world to prevent tuberculous disease. This vaccine rarely causes disease in healthy children, but dissemination complicating the vaccination of previously presumably healthy infants and children has been well described [1, 2]. BCG is also used as adjuvant therapy for treatment of superficial bladder cancer. A previous report of nosocomial infection due to *M. bovis* BCG in 2 children who developed BCG meningitis implicated contamination of chemotherapy solutions that were reconstituted under the same biosafety hood as the BCG vaccine used for bladder cancer therapy [3]. We report 3 more instances of similar nosocomial transmission of BCG vaccine infections, including 1 patient with localized CNS infection and 2 with disseminated disease. One of the patients with dissemination exhibited an uncommon manifestation of disseminated tuberculosis, which had previously been better described in patients infected with *Mycobacterium tuberculosis*. We also present evidence of transmission to susceptible health care workers (HCWs) by respiratory spread from 1 of these patients.

Case Reports

**Patient 1.** A 2 1/2-year-old boy with trisomy 21 and acute megalokaryocytic leukemia in remission presented with a history of 16 days of fever. Irritability and decreased appetite were noted on the 16th day. His other medical problems included pulmonary hypertension requiring oxygen therapy, successfully repaired tetralogy of Fallot, right bundle branch block, hypothyroidism, and gastroesophageal reflux. He was admitted for work-up of fever of unknown origin.

Physical examination revealed an awake, alert, and responsive toddler. The chest was clear to auscultation. His heart rate and rhythm were regular with a grade 2/6 systolic ejection murmur. His abdomen was soft and nontender. Admission laboratory studies included the following values: WBCs, 11,300/μL (75% polymorphonuclear cells, 4% band forms, 12% lymphocytes, and 9% monocytes); hemoglobin, 9.1 g/dL; hematocrit, 26.8%; platelets, 373,000/μL; alanine transaminase (ALT), 127 U, and aspartate transaminase (AST), 54 U. Chest radiography revealed bilateral coarse granular opacities.

PPD (5 TU) was reactive at 72 h (15 mm induration). Bone marrow evaluation revealed epithelioid granulomas and a negative acid-fast bacilli (AFB) staining. Examination of smears of a bronchoalveolar lavage (BAL) specimen were negative for both AFB and *Pneumocystis carinii*. Smears and cultures of 3 early morning gastric aspirates were negative for AFB. Examination of an open lung biopsy specimen revealed granulomas and AFB. Repeated chest radiography showed right-sided...
pleural effusion. Follow-up films revealed diffuse bilateral parenchymal disease and bilateral pleural effusions. Initial empiric therapy included isoniazid (10 mg/kg/d), rifampin (10 mg/kg/d), pyrazinamide (30 mg/kg/d) and ethambutol (20 mg/kg/d). Culture of the lung biopsy specimen yielded a mycobacterium identified as *M. tuberculosis* (MTB) complex, by use of DNA-RNA hybridization assay (Gen-Probe, San Diego, CA), that was subsequently speciated as *M. bovis*. The isolate was susceptible to isoniazid at 0.2 μg/mL, rifampin at 0.5 μg/mL, and ethambutol at 4 μg/mL, but resistant to pyrazinamide at >99 μg/mL. After 8 weeks of therapy, pyrazinamide and ethambutol were discontinued. The patient was treated with isoniazid and rifampin for 20 months. Follow-up 20 months later showed no evidence of recurrent disease. Family contact study revealed that the PPDs (5TU) of his father and siblings, ages 6, 10, and 12, were nonreactive. His mother had been PPD reactive several years earlier and had received 1 year of preventive treatment with isoniazid.

**Patient 2.** A 13-year-old girl with acute lymphocytic leukemia (ALL), neutropenia, thrombocytopenia, and anemia presented with a fever and rash in early January 1995. She was in the maintenance phase of chemotherapy for relapsed ALL and had been in remission for 32 weeks. She presented with a 3-week history of increasing cough. Three days before admission she developed a rash on her arms and trunk. She was admitted to the hospital when her temperature increased to 38.8°C. At presentation, physical examination revealed a temperature of 38.4°C, pulse of 133/min, and weight of 36.8 kg. She was a tired appearing, pale, awake, cooperative teenager. Skin examination showed multiple erythematous pigmented lesions over the arms, abdomen, and lower extremities, with 1 or 2 crustified pigmented lesions over her anterior tibias. These lesions were 1–2 cm in diameter, circular, and nontender to palpation (figure 1). Chest examination revealed decreased breath sounds at the lung bases, and abdominal examination revealed a palpable spleen tip.

Laboratory evaluation on admission revealed the following values: WBCs, 300/μL (26% polymorphonuclear cells, 2% band forms, 65% lymphocytes, 4% monocytes, 3% eosinophils, and an absolute granulocyte count of 84); hemoglobin, 10.5 g/dL; platelet count, 56,000/μL; ALT, 48 U; AST, 55 U; and lactate dehydrogenase (LDH), 297 U. Chest radiography revealed diffuse reticular nodular infiltrates in both lungs, with a thin-walled cavitary lesion in the left lung field.

She was admitted to the hospital for febrile neutropenia and treated with initial empiric therapy of iv cefazidime and vancomycin. Histopathologic examination of biopsies of the skin lesions showed 4+ AFB without evidence of granuloma formation. Evaluation of BAL and sputum specimens revealed 3+ AFB and 4+ AFB, respectively, prompting standard respiratory isolation for AFB. Evaluation of a bone marrow aspirate revealed pancytopenia with no relapse of her leukemia. The CSF contained only 1 WBC, and the protein and glucose concentrations were normal. Therapy was changed to that with isoniazid (300 mg q.d.), rifampin (600 mg/d, or 16 mg/kg/d), pyrazinamide (1 g q.d. or 27 mg/kg/d), ethambutol (600 mg/d, or 16 mg/kg/d), and clarithromycin (500 mg b.i.d.). The sputum remained AFB smear-positive for 2 weeks. Serology for antibodies to HIV remained negative. Cultures of the skin biopsy and BAL specimens yielded AFB at 9 and 12 days, respectively. The isolates were MTB complex positive by use of DNA-RNA hybridization assay (Gen-Probe) and were subsequently identified as *M. bovis*. Cultures of the patient’s sputum, bone marrow, and blood all yielded heavy growth of *M. bovis*. The isolates were susceptible to isoniazid at 0.2 μg/mL, rifampin at 0.5 μg/mL, ethambutol at 4 μg/mL, streptomycin at 1 μg/mL, amikacin at 0.3 μg/mL, and ciprofloxacin at 0.6 μg/mL; however, they were resistant to pyrazinamide.

Evaluation for exposure to tuberculosis revealed that the father, mother, and 11-year-old sibling all had negative skin tests prior to and 3 months after the patient’s diagnosis of miliary tuberculosis. The patient had no history of ingestion of unpasteurized dairy products, and her travel history was significant only for a previous visit to Guam for 1 month, 7 years before this illness.

Clinical hepatitis developed after 10 days of treatment. Therapy with rifampin was discontinued and replaced with ciprofloxacin, 500 mg b.i.d. Therapy with isoniazid was discontinued at 5 weeks and replaced with amikacin (22 mg/kg/d b.i.d.). During hospitalization, the liver function tests increased dramatically with a peak ALT level of 229 U, AST level of 420 U, and LDH of 1216 U. Upon review of the infection’s susceptibility to different antimicrobials, amikacin and pyrazinamide were discontinued, and the patient was eventually treated with ethambutol (25 mg/kg/d) and ciprofloxacin (500 mg b.i.d.) for 6 months. During this time, the patient’s chest radiograph showed improvement, but in January 1996 she began to experience cough accompanied by fevers, decreased appetite, and increased fatigue. She was admitted to the hospital in March 1996 for 7 weeks, during which time her sputum and bronchoscopy smears were once again positive for AFB. The susceptibility of the *M. bovis* recovered from the bone marrow was similar to that for the previous isolate. Rifampin, streptomycin, and ethionamide were added to the current regimen. Streptomycin and ethionamide were discontinued after 2 months. Treatment was continued with rifampin, ethambutol, and ciprofloxacin. A gastrostomy tube was placed for nutritional support.

In November 1996 her condition deteriorated; she developed respiratory distress, pneumonia, hypotension, coagulopathy, and heart failure before she died later that month. Postmortem examination showed congestive heart failure, diffuse bilateral pneumonia with multiple caseous necrotizing granulomas, bilateral pleural effusions, pericardial effusion, and multiple necrotizing granulomas of subcarinal, peribronchial, and abdominal lymph nodes. Evaluation of the bone marrow dem-
onstrated reactive hypercellular marrow with no evidence of relapse of ALL.

**Patient 3.** A 6-year-old boy with ALL presented with fever of 3 weeks’ duration associated with 10 days of knee pain. He was receiving maintenance chemotherapy with mercaptopurine (6-MP) and methotrexate, as well as *P. carinii* pneumonia prophylaxis with trimethoprim sulfamethoxazole. Medical evaluation revealed a posterior spinal epidural abscess from L2 to L5, and an emergent total laminectomy was performed. The tissue abscess was AFB smear-positive and *M. bovis* was subsequently recovered. This case has been reported elsewhere [4].

**Methods**

*Mycobacterial speciation.* Isolates from all patients were identified initially as *M. bovis* at the University of California San Diego
Figure 2. Pulsed-field gel electrophoresis of *Mycobacterium bovis* Bacille Calmette-Guerin (BCG) isolates. DNA fragments were separated after digestion with *Dra*I (A), *Ase*I (B) and *Spe*I (C). Sizes of DNA markers are indicated at the left of each panel. Lane 1, Molecular weight marker—48.5 kbp λ concatemer; lane 2, patient 2; lane 3, patient 1; lane 4, BCG-Tice (CDC); lane 5, BCG-Connaught; lane 6, BCG-Tice (Organon Teknika); lane 7, BCG-Tice (Organon-Teknika, hospital lot).

Genomic DNA was restricted with the low-frequency-cleavage restriction endonucleases, *Dra*I (5’-TTTAAA-3’), *Ase*I (5’-ATTAAT-3’), *Spe*I (5’-ACTAGT-3’) and *Xba*I (5’-TCTAGA-3’). PFGE of the digested samples was performed by use of CHEF–Dynamic Regulation (DR) II system (BioRad Laboratories). Agarose plugs were embedded in 1% low-endosmosis agarose gels and electrophoresed at 14°C in TBE buffer (25 μM Tris, 25 μM boric acid, 0.5 μM EDTA) at 6 V/cm. The ramped pulse times varied according to the enzyme used; for the *Dra*I digestion it was 60–70 s for 4 h and then 5–15 s for another 15 h; for the *Ase*I and *Xba*I digested DNAs, 3–15 s for 20 h. DNA size standards consisting of concatemers of bacteriophage λ starting from 48.5 kbp were included in each gel. Finally, the gels were stained with ethidium bromide, visualized on an ultraviolet transilluminator, and photographed.

Contact investigation. A tuberculosis contact investigation and a BCG preparation and administration investigation were performed.

Results

**PFGE.** The PFGE patterns produced by 4 different restriction enzymes for the isolates from patients 1 and 2 were identical to one another and to the BCG Tice strains, BCG Tice (source CDC) (obtained from Richard Wallace, University of Texas Medical Center, Tyler, TX) and TICE BCG (as licensed by Organon Teknika for bladder cancer chemotherapy percutaneous vaccination) and BCG Connaught (source CDC) (another BCG strain licensed in the United States for bladder cancer chemotherapy; obtained from Richard Wallace; figure
2). Both strains obtained from Richard Wallace have been previously described by Zhang et al. [7]. The isolate from patient 3 demonstrated a single band-shift after DraI restriction but was identical to the same BCG Tice strains after digestion with the 3 other enzymes.

**Contact investigation.** Evaluation of clinic records and family interviews revealed that these 3 patients had no prior contact with one another, either in the medical setting or socially. Soon after learning that the 13-year-old patient with active disease had positive AFB sputum smears, a contact investigation was initiated among the 46 health care workers (HCWs) who provided care to her. Five of the HCWs were known to have had previously positive PPD reactions. All had negative chest radiographs on follow-up. Forty-one HCWs with previously negative skin tests were skin tested with PPD (5 TU). Two (5%) were positive and considered PPD converters. The first of these was a nurse on the pediatric ward where the patient was admitted and had had direct contact with her. She had a negative PPD, documented in June of 1994. In late February 1995, her Mantoux skin test produced a 17-mm induration, although her chest radiograph was normal and she did not develop any evidence of tuberculous disease. The second PPD converter was a nurse who worked in the pulmonary clinic where this patient’s sputum was induced. His PPD was nonreactive in 1990. In February 1995, his Mantoux skin test measured 28 mm induration. His chest radiograph remained normal without evidence of old or active tuberculosis.

**BCG preparation investigation.** Before July 1994, BCG used for bladder instillation and chemotherapy solutions was prepared under a single biologic safety hood in the pharmacy. BCG and chemotherapy solutions were prepared on the same day and usually in the early morning. An extensive review of the procedure used to prepare BCG failed to reveal any deviation from standard operating procedures and followed the manufacturer’s recommendations. All 3 of our patients received chemotherapy that was prepared before July 1994. The commercial strain of BCG used during this period was TICE BCG (Organon Teknika). Since July 1994, BCG used for bladder instillation has been prepared under a separate biologic hood devoted to this use only. No chemotherapy solutions have been prepared under the same hood as BCG since July 1994.

**Discussion**

We have described 3 immunocompromised children with no identifiable exposure to active tuberculosis who developed serious tuberculous disease while receiving cancer chemotherapy for treatment of leukemia. After *M. bovis* infection was recognized as the cause of disease, an epidemiological investigation was undertaken to find the source of infection. Results of the investigation strongly suggest nosocomial transmission of *M. bovis* BCG, given that the PFGE profiles of the patients’ isolates were virtually identical to those of the 2 BCG vaccine strains licensed in the United States for bladder cancer chemotherapy. All 3 mycobacterial isolates were initially identified as *M. bovis* by use of standard biochemical tests, and were subsequently confirmed to be BCG strains by both gas-liquid chromatography and MPTR-PCR.

Connaught and Tice BCG are live attenuated cultures prepared from bacillus of Calmette-Guerin strain of *M. bovis*. In the United States, these 2 BCG strains have been widely used in the treatment of carcinoma in situ of the bladder by inducing a granulomatous reaction at the local site of administration. In many parts of the world, BCG vaccine is also administered percutaneously to lower the risk of serious complications of primary tuberculosis in children [8]. A recent review of disseminated BCG infections after vaccination described methods of identification of BCG, diagnostic criteria for disseminated BCG disease, and treatment of disseminated disease [1]. *M. bovis* BCG has been associated with pulmonary and disseminated disease [2].

By using specific restriction endonucleases and PFGE to determine the epidemiology of these infections, we showed that isolates from 2 of the patients were identical strains, and identical to both the Tice and Connaught strains of BCG that are used for bladder chemotherapy. The other patient’s isolate was very similar to these 2 BCG strains except for a single band-shift with *Dra*I. PFGE after low-frequency-cleavage endonuclease restriction has been used successfully by others to show that tuberculous lesions in immunocompromised patients were caused by BCG strains that were identical to the vaccine strain previously administered to each patient [3, 7]. The 2 patient strains, the Organon Teknika sample, the BCG Tice (source CDC) and BCG Connaught (source CDC) were identical to one another with use of all 4 restriction enzymes. The PFGE patterns of the latter 2 strains were previously shown to be indistinguishable when restricted by the 4 different restriction enzymes [7]. The *Dra*I pattern of these 2 strains was different from those of BCG Brazilian, BCG Copenhagen, BCG Danish, BCG French, BCG Glaxo, BCG Japanese, BCG Moreau, BCG Pasteur, BCG Russian, and BCG Swedish [7]. Therefore, the technique can distinguish the BCG Tice and Connaught from many other BCG strains.

These data are consistent with the conclusion that patients 1 and 2 were infected with the BCG Tice from Organon Teknika, which was the specific strain used at the institution where these patients were diagnosed. The source of infection in patient 3 was problematic because the *Dra*I pattern of his isolate revealed a band of a different size than in the pattern of the other strains. This size difference could be accounted for by loss of a *Dra*I site, insertion of a mobile element, or chromosomal rearrangement. Such changes were noted previously with different lots of BCG Danish, BCG Glaxo, and BCG Pasteur [7]. It is of interest that patients 1 and 2 manifested their infections within 1 month of each other in 1994, but patient 3 was infected nearly 3 years earlier. Although we could not obtain definitive
evidence, it is possible that different lots of Tice BCG from Organon Teknika were used during these 2 periods.

The exact mechanism by which M. bovis BCG was transmitted to these 3 children is unclear. Direct inoculation seems unlikely, since this would require administration of the wrong chemotherapeutic agent into 3 different patients under stringent pharmacy protocols. Human-to-human transmission via aerosol is a possibility for patients 1 and 2, but a careful review of their clinic records and interviews with the families failed to reveal any contact between the 2 patients inside or outside the hospital setting. More likely, M. bovis was transmitted by inadvertent aerosol contamination of chemotherapeutic drugs mixed under the same biologic hood as that used for preparation of the BCG, despite the absence of evidence for breaks in sterile technique or deviations from the manufacturer’s specifications. However, we cannot exclude the possibility that the chemotherapeutic agents were inoculated by contaminated gloves or by other equipment used to prepare these agents after preparation of BCG under the same hoods or by the same personnel. In a similar investigation, no definitive route of transmission was identified for the 2 children reported with BCG meningitis, but the investigators also postulated aerosol transmission [3]. It is of interest that another 6-year-old girl who was being treated with intrathecal chemotherapy for acute lymphocytic leukemia developed a BCG brain abscess. No source for this infection was ever detected [9]. A recent letter and comment reviewed the remarkable similarities of 5 reported cases of M. bovis BCG meningitis in immunocompromised patients and speculated on the route of transmission [10, 11]. In another report [12], 4 immunocompromised patients developed miliary tuberculosis due to M. bovis BCG after receiving cancer chemotherapy. All the patients received their chemotherapy in the same oncology room, and the same team administered weekly intravesical BCG to patients with bladder cancer. By use of restriction fragment length polymorphism (RFLP) techniques, the 3 clinical isolates of M. bovis BCG were shown to be identical to the BCG strain that was used to treat the bladder cancer patients.

One of the patients we reported had an uncommon complication of miliary disease. This patient developed disseminated skin lesions, and these were helpful in establishing the diagnosis of tuberculosis disease. Although this particular dermatologic condition has been described as a manifestation of disseminated M. tuberculosis [13], there is only 1 report of skin involvement beyond the original BCG vaccine inoculum site in idiopathic disseminated BCG disease in children [14]. However, a description of the skin lesions was not provided in that report. This dermatologic manifestation of tuberculosis has been referred to as tuberculosis cutis miliaris disseminata. In contrast to other forms of cutaneous tuberculosis, it is most often associated with negative tuberculin reactions and a poor prognosis.

The patient with skin lesions also had active pulmonary disease with heavily AFB-positive sputum, prompting a HCW exposure work-up. Of 41 HCWs with previously negative TB skin tests, 2 (5%) were positive and were considered PPD converters. One HCW had a clearly documented recent conversion and worked on a pediatric ward, which is considered to be a low risk area for tuberculosis. The other HCW had a 5-year interval between skin tests and worked in the pulmonary clinic, which is a higher risk area for exposure to tuberculosis in the hospital. This second HCW could very well have been infected through another unrelated occupational exposure.

There are now at least 5 cases of nosocomial transmission of BCG vaccine strain infection to immunocompromised children, most likely through aerosol contamination of cancer chemotherapy prepared under the same biosafety hood. In addition, we have provided evidence for probable human-to-human transmission via the pulmonary route from an infected patient to a HCW, underscoring the infectious potential of this attenuated vaccine strain. Definitive proof of transmission of M. bovis BCG would require isolating the organism from the infected HCW for comparison to the index case.

Nosocomial transmission of M. bovis BCG through preparation and administration of cancer chemotherapy is an important route of infection, which may not be known or recognized by medical and pharmacy personnel. Efforts to prevent future nosocomial transmission of BCG to immunocompromised patients should include a careful review of infection control practices in hospital and clinic pharmacies that prepare both BCG vaccine and infusion solutions, as well as the use of separate bioguard safety hoods until the development of self-enclosed systems for the safe reconstitution of BCG vaccine.

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References


