Recurrent, Disseminated *Mycobacterium marinum* Infection Caused by the Same Genotypically Defined Strain in an Immunocompromised Patient

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Received 15 January 1999/Returned for modification 11 March 1999/Accepted 27 May 1999

An 81-year-old male with myasthenia gravis developed a cutaneous infection with *Mycobacterium marinum*, which apparently resolved following local heat therapy. Five months later, the patient developed new skin lesions and pancytopenia. *M. marinum* was isolated from his bone marrow. Pulsed-field gel electrophoresis was performed to determine if the skin and bone marrow isolates were clonally related. Digestion of the genomic DNA with the restriction enzymes *Spe*I and *Asel* yielded indistinguishable banding patterns. An epidemiologically unrelated control strain showed significant banding differences. The results suggest that the patient's recurrent, disseminated infection was due to recrudescence of his initial infection rather than reinfection by another strain.

*Mycobacterium marinum* is a photochromogenic mycobacterium that is ubiquitous in the aquatic environment (7). First isolated in 1926 by Aronson (1) from saltwater fish that had died in the Philadelphia Aquarium, it was not recognized as a cause of human disease until 1951, when Norden and Linnel (11) isolated the organism from granulomatous skin lesions in swimmers. Infection typically arises when traumatized skin comes into contact with infected water in swimming pools, aquariums, oceans, or lakes. Because of the organism's optimal growth at 30 to 32°C, infection is usually limited to the skin in the peripheral, cooler parts of the body, particularly the hands. The typical lesion is a single nodule that occasionally progresses along the lymphatics in a sporotrichoid fashion (7). Less commonly, patients develop deeper infections, involving tendon sheaths or periartriolar tissues (13), and, rarely, septic arthritis or osteomyelitis can ensue (2, 4). Disseminated cutaneous lesions have also been described to occur in both immunocompetent (8, 17) and immunocompromised patients (5, 6). Because of the organism's poor growth at 37°C, however, systemic dissemination is extraordinarily rare and has only been reported to occur in immunocompromised patients (9, 12, 14).

We present a case of systemically disseminated *M. marinum* infection in an immunocompromised patient, who had been diagnosed 5 months earlier with cutaneous disease that had apparently resolved following local heat therapy. The second isolate was recovered from the patient's bone marrow, which was cultured after the patient became pancytopenic. Pulsed-field gel electrophoresis (PFGE) was performed to determine if the skin and bone marrow isolates were clonally related.

Case report. An 81-year-old white male presented with a painful, erythematous plaque on the dorsum of his left forearm. He had a 10-year history of myasthenia gravis, for which he was taking prednisone (40 mg every other day) and azathioprine (275 mg/day). He was seen by a dermatologist, who felt the lesion probably represented chronic erysipelas, and was treated with ciprofloxacin for 1 month. Although there was some initial improvement, the lesion persisted and began to ulcerate. A separate ulcer developed over the metacarpophalangeal joint of his left index finger. A biopsy was performed, and this revealed a lymphohistiocytic infiltrate in the dermis with necrosis and acute inflammation. The findings were most suggestive of an infectious process, but special stains for fungi, bacteria, and acid-fast organisms were negative. The skin lesion was cultured for mycobacteria on Lowenstein-Jensen and 12B Bactec media at 30 and 37°C. Growth occurred in Bactec media after 8 days at 30°C. Initial testing by using commercial DNA-RNA hybridization probes (Gen-Probe, San Diego, Calif.) revealed negative results for *M. tuberculosis* complex, *M. avium* complex, *M. gordonae*, and *M. kansasii*. By Runyon typing, the organism was found to be a photochromogen that preferred growth at 30°C compared to 37°C. Biochemical testing (16) showed positive reactions for urease, Tween hydrolysis (both 5- and 10-day readings), and pyrazinamidase activity (both 4- and 7-day readings). Negative tests included nitrate reduction (tube test), arylsulfatase (3-day test), and heat-stable catalase (68°C), and the organism did not grow on MacConkey agar without crystal violet (5- and 11-day readings). The results of the biochemical tests were indicative of *M. marinum* and were confirmed by gas-liquid chromatographic analysis of cellular fatty acids (18).

Following the diagnosis, it was learned that the patient maintained a small aquarium at home. Because of gastrointestinal complaints while taking ciprofloxacin, the patient refused antibiotic therapy, but he agreed to wrap his arm daily with a heating pad. Three months later, when the patient was admitted to the Johns Hopkins Hospital with a myasthenic crisis, the skin lesions had almost completely resolved except for some residual postinflammatory hyperpigmentation. At the time of hospitalization, he did note some arthritic pain in his left hand, but the pain was easily relieved by taking ibuprofen. After 5 courses of plasma exchange therapy, the patient's myasthenia gravis stabilized and he was sent home.

Three weeks after being discharged, the patient was readmitted to the hospital because of marked, painful swelling of his right arm. Examination revealed a 30- by 8-cm area of erythematous induration with focal ulceration and blister formation along the dorsal and radial sides of the forearm and the...
medial side of the upper arm. The patient was treated with clindamycin and gentamicin but was switched to intravenous vancomycin after bacterial cultures from the lesion grew methicillin-resistant Staphylococcus aureus. Antibiotics did not cause the lesion to regress, however, and a similar 5- by 5-cm area developed around the antecubital fossa of the left arm. Although 5 months had elapsed since the initial skin biopsy-based diagnosis, the patient was also placed on intravenous doxycycline because of concerns of M. marinum infection. Mycobacterial cultures from the right arm, however, were negative.

The patient gradually became pancytopenic and developed rising levels of creatinine and elevated liver function tests. Because of his pancytopenia, azathioprine was discontinued, and bone marrow biopsy and cultures were performed. The biopsy showed mild hypocellularity with a slight lymphocytosis. Mycobacterial cultures of bone marrow specimens incubated at 37°C were positive at 3 weeks. Before the isolate was identified, however, the patient became encephalopathic and died. The results of biochemical tests performed on the bone marrow isolate were identical to the results from the skin isolate, confirming that the organism grown from the bone marrow was in fact M. marinum. Gas-liquid chromatographic analysis of cellular fatty acids also gave an identification as M. marinum.

To determine whether the skin and bone marrow isolates were clonally related, PFGE was performed. The two isolates of M. marinum from the patient were compared to M. marinum ATCC 927 in order to demonstrate the ability of PFGE to discriminate a potentially related organism from an organism known to be unrelated epidemiologically. PFGE was performed with some modification of established protocols (15, 20). In a biosafety level 3 facility, the M. marinum isolates were grown for 2 weeks at 30°C in 10 ml of Middlebrook 7H9 broth (BBL, Cockeysville, Md.) supplemented with 0.1% Tween 80. Before DNA extraction, ethambutol, a cell wall-active agent, was added to 1 μg/ml, a concentration expected to be subinhibitory. Cultures were further incubated about 16 h. Cells were harvested by centrifugation and resuspended in 1 ml of suspension reagent (1 M NaCl, 10 mM Tris-HCl [pH 7.6]). Cell suspensions were mixed 1:1 with molten 1.6% InCert Agarose (FMC, Rockland, Maine) and pipetted onto glass slides. Several plugs, approximately 1 by 5 by 10 mm, were cut from the slide by using a microscope coverglass and placed into about 5 ml of lysis solution (1 M NaCl, 100 mM Na₂EDTA [pH 7.5], 0.5% Brij 58, 0.2% deoxycholate, 0.5% sodium lauryl sarcosine, and 5 mg of lysozyme per ml [Sigma, St. Louis, Mo.]). Plugs were incubated overnight at 37°C. Lysis solution was replaced with ESP reagent (0.5 M EDTA [pH 9], 1% sodium lauryl sarcosine, 500 mg of proteinase K per ml [Sigma]), and the plugs were incubated overnight at 50°C. Plugs were washed three times at 37°C for 1 h each time with TE (10 mM Tris-HCl [pH 7.4] and 0.1 M Na₂EDTA [pH 8]).

For DNA macrorestriction, the agarose plugs were placed in a solution containing 200 μl of deionized water, 25 μl of 10× buffer, and 20 U of SpeI or AseI (New England Biolabs, Beverly, Mass.), supplemented with bovine serum albumin as indicated by the manufacturer, and incubated for 4 h at 37°C. After a brief wash with TE, inserts were loaded into 1% agarose gels (Bio-Rad, Hercules, Calif.). PFGE was performed with a Bio-Rad CHEF DRII apparatus with an increasing pulse time of 1 to 20 s over a total run time of 22 h in 0.5× TBE (1 M Tris-HCl, 0.9 M boric acid, 10 mM Na₂EDTA [pH 8]) at 200 V. A well-characterized strain of Enterococcus faecalis, OGIRF, was digested with NotI to serve as the molecular weight marker (10). The gel was stained with ethidium bromide for UV transillumination and Polaroid photography.

Restriction profiles of the isolates’ genomic DNA obtained by SpeI digestion yielded 20 to 25 fragments ranging in size from less than 20 to approximately 280 kbp (Fig. 1). The skin isolate (lane 2) and bone marrow isolate (lane 3) demonstrated indistinguishable banding patterns, whereas an epidemiologically unrelated, control isolate of M. marinum (lane 4) showed significant (>5) multiband differences. A second restriction digestion with AseI was also performed on the same gel. Once again, the skin isolate (lane 5) and bone marrow isolate (lane 6) displayed indistinguishable banding patterns. The control isolate (lane 7) showed at least four band differences.

Discussion. M. marinum infection in otherwise healthy hosts can be self-limiting and disappear after several months, or it can be treated with a variety of antimicrobial drugs, including trimethoprim-sulfamethoxazole, rifampin and ethambutol, or doxycycline (7). Alternatively, local heat therapy has also been used with good results. Topical or local injection of steroids, however, is contraindicated because it frequently causes exacerbation of the infection and leads to greater difficulty in curing the patient (3, 19).

In contrast to the situation with immunocompetent hosts, treating M. marinum infection in immunocompromised patients can be very difficult (12, 14). One reason for the difficulty could in part be related to delays in accurate diagnosis. There is frequently a substantial lag time between the appearance of the skin lesion and the correct diagnosis, ranging from a few weeks to several years (7). Clinical misdiagnosis is common, and organisms are rarely identified on acid-fast stains of biopsy material. Such delays in diagnosis can lead to local spread of the infection, especially in immunocompromised hosts. Once the correct diagnosis is made and appropriate antimicrobial therapy is started, however, M. marinum infections are still difficult to control in immunosuppressed patients, necessitating many months of treatment with antibiotics, and even then, the results can be disappointing.

The present case confirms the difficulty in treating M. marinum infections in immunocompromised patients. Local heat
therapy apparently cleared the cutaneous infection, but it might not have prevented spread to deeper tissues. The patient's joint pain in the left hand could have represented involvement of the joint space. A few weeks later, he had disseminated cutaneous lesions involving the right and left arms, and the systemic dissemination to the bone marrow. Despite intravenous doxycycline, the patient died in multiorgan system failure, presumably from his *M. marinum* infection.

The skin and bone marrow isolates were the same genotypically defined strain based on the identical patterns generated by PFGE after macrodigestion. Significant multiband differences were seen with an epidemiologically unrelated control strain. From a clinical standpoint, the findings suggest that the second isolate probably represented recrudescence of the patient's initial infection rather than reinfection by another strain of *M. marinum*. The most likely source of infection was the patient's aquarium, but unfortunately, cultures from the aquarium itself were not performed. It is unknown how often the patient's aquarium, but unfortunately, cultures from the aquarium itself were not performed. It is unknown how often the patient had contact with the aquarium in the intervening 5 months between the two positive cultures. The possibility that the patient was reinfected by the same strain of *M. marinum* after further contact with the aquarium therefore cannot be entirely excluded.

REFERENCES