Systemic Infection with *Mycobacterium genavense* Following Immunosuppressive Therapy in a Patient Who Was Seronegative for Human Immunodeficiency Virus

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We describe, to our knowledge, the first case of disseminated *Mycobacterium genavense* infection in a patient who was seronegative for human immunodeficiency virus. The patient, a 47-year-old woman, had been previously treated with immunosuppressive drugs for 9 months to control an unclassified immunologic disorder characterized by intermittent fever and inflammatory pulmonary, hepatic, and dermal infiltrates. Antemortem and postmortem examinations revealed the presence of numerous mycobacteria in the bone marrow, spleen, kidneys, and lungs; these organisms failed to grow in vitro and were identified as *M. genavense* by 16S rRNA gene sequencing. This case illustrates that systemic *M. genavense* infections are not restricted to patients with AIDS but can also occur in otherwise immunocompromised patients.

Tuberculous and nontuberculous mycobacteria are frequent causes of opportunistic infections in patients with inherited or acquired defects of the cellular immune response. In the past, generalized infections with *Mycobacterium genavense*, a new member of the genus *Mycobacterium* characterized by extremely slow and variable growth in vitro [1, 2], were exclusively reported among patients with AIDS [3, 4]. We describe a case of systemic infection with *M. genavense* in an HIV-seronegative woman who was treated with immunosuppressive agents for a presumed immunologic disorder.

**Case Report**

A 47-year-old female bakery shop assistant was well until June 1994, when she first presented to her family doctor with a nonproductive cough, a sore throat, and rhinitis. Bronchospasm was detected, and she was treated systemically with prednisolone (15 mg/d, then 7.5 mg/d) for 3 weeks. During the course of therapy, she developed large (≤5–10-cm), livid, nodular-to-confluent dermal infiltrations on both thighs and upper arms, which were diagnosed as erythema nodosum. In August 1994 she was admitted to a local hospital because the cough persisted and she had developed daily intermittent fevers (temperature, ≤39.5°C) with chills.

Laboratory studies on admission revealed the following values: hemoglobin, 10.6 g/dL; hematocrit, 33%; WBC count, 3,900/mm³ (65% neutrophils, 13% band forms, 17% lymphocytes, 3% eosinophils, and 1% basophils); platelet count, 409,000/mm³; erythrocyte sedimentation rate, 108 mm/h; and C-reactive protein, 22.9 mg/dL. The total serum protein level was 6.5 g/dL (48.7% albumin, 16.9% α₂-globulins, and 20.9% γ-globulins). Repeated blood cultures performed with the Bactec NR730 system (Becton Dickinson, Heidelberg, Germany) remained sterile. Mycobacteria were not detected microscopically, and cultures of sputum, gastric contents, and bronchoalveolar lavage fluid performed with use of Löwenstein-Jensen medium and the Septi-Check acid-fast-bacilli culture system (Becton Dickinson) were negative.

An intracutaneous tuberculin test was negative. Serologies were negative for antibodies to HIV-1 and HIV-2, cytomegalovirus, Epstein-Barr virus, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Brucella* species, *Borrelia* species, *Mycoplasma* species, and *Coxiella burnetii*. The patient’s serum was also negative for rheumatoid factors and for autoantibodies to cytoplasm, mitochondria, nuclei, and extractable nuclear antigens. A chest radiograph and a CT scan showed confluent perihilar infiltrations in the middle and lower regions of both lungs, which extended into the peripheral interstitium. Findings on ultrasonograms of the liver, spleen, and heart were normal, as were findings on a transesophageal echocardiogram.

Histological analysis of a tranbronchial biopsy specimen revealed a subtle inflammatory process in the lung, without granulomata or findings typical of sarcoidosis. Examination of the bone marrow showed reactive enhancement of hematopoiesis but no signs of malignancy. The skin lesions were characterized by a dermal infiltration with histiocytes, neutrophils, and eosinophils, compatible with interstitial granulomatous dermatitis. Examination of a liver biopsy specimen revealed a prominent perportal inflammatory reaction with little hepatocytic necrosis.

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Because there was no evidence of an infection or a malignant process, the inflammation of multiple organs was interpreted as an unclassified systemic autoimmune disorder that was treated with prednisolone (60 mg/d). This treatment led to immediate resolution of the fever and striking regression of the skin lesions and pulmonary infiltrates. The patient was discharged at the end of August 1994 with instructions to continue taking prednisolone (10 mg/d). A relapse of the previous symptoms and signs occurred 8 weeks later and was controlled by therapy with azathioprine (100 mg/d) plus prednisolone (60 mg/d).

In January 1995 the patient was again hospitalized because of a high fever (temperature, $<40^\circ$C), extensive oropharyngeal candidiasis and dysphagia, livid dermal infiltrates on both thighs and lower arms, and general fatigue. The WBC count was 3,600/mm$^3$ (93% neutrophils with 22% band forms, signs of disease [9, 10], systemic infections with esophageal candidiasis and dysphagia, livid dermal infiltrates immunocompetent, HIV-seronegative individuals [8] and has requiring intensive care with intubation and repeated transfusions of systemic mycobacteriosis and fever, and she developed hypotension, increasing respiratory immunosuppressants, perhaps during an undiagnosed bacterial coplanin, fluconazole, G-CSF, prednisolone (20 mg/d), and the first hospitalization of the patient.

As bone and urine cultures remained negative and the pulmonary infiltrates became more prominent, treatment with ganciclovir was begun. Although ganciclovir was later replaced by foscarinet and treatment with azathioprine was discontinued, the patient developed pancytopenia. Despite the administration of antibacterial therapy (piperacillin/tazobactam, gentamicin, and rifampin) and of granulocyte colony-stimulating factor (G-CSF; 300 $\mu$g/d) and prednisolone (100 mg/d), the high fever and pancytopenia (400–1,200 WBCs/mm$^3$ with 80%–90% neutrophils, ≤30% band forms, and 3%–11% lymphocytes) did not resolve.

In March 1995 the patient was transferred to the medical department of our university. Treatment with imipenem, ticloplalin, fluconazole, G-CSF, prednisolone (20 mg/d), and dipyridone was started immediately. She continued to have a fever, and she developed hypotension, increasing respiratory insufficiency, diffuse bleeding episodes, and kidney failure requiring intensive care with intubation and repeated transfusions. Numerous acid-fast bacteria were seen in a bone marrow biopsy specimen, bronchial secretions, and bronchoalveolar lavage fluid, and a diagnosis of systemic mycobacteriosis was made. A ribosomal RNA target amplification assay [5] (Genprobe, San Diego) revealed that all specimens were negative for M. tuberculosis-specific nucleic acid. In addition, Pneumocystis carinii cysts were found in the lavage sediments. Two days after these results were obtained, the patient died of a severe infratentorial hemorrhage.

Postmortem examination revealed masses of histiocytes and intracellular mycobacteria in the bone marrow as well as large numbers of mycobacteria in the lungs, spleen, and kidneys. Mycobacterial cultures of tissue specimens, which were performed on Löwenstein-Jensen medium and Stonebrink medium with use of the Septi-Chek system and in BACTEC 12B bottles (Becton Dickinson), remained negative for an observation period of 3 months. Ziehl-Neelsen staining did not reveal any mycobacteria in the CNS, liver, or lymph nodes. PCR amplification of DNA extracted from the patient’s spleen tissue with primers specific for the 16S rRNA of the Mycobacterium genus [6] yielded a product that was 100% identical to the published DNA sequence coding for the 16S rRNA of M. genavense [2].

**Results and Discussion**

During the past 5 years, M. genavense has been recognized as an important opportunistic pathogen in patients with AIDS (i.e., those with CD4 lymphocyte counts of $\leq50$/mm$^3$). The most commonly involved organs included the small intestine, bone marrow, spleen, liver, and lymph nodes [3, 7]. Although M. genavense has been found to colonize the intestines of immunocompetent, HIV-seronegative individuals [8] and has been repeatedly isolated from pet dogs and birds with various signs of disease [9, 10], systemic infections with M. genavense have not yet been reported in HIV-seronegative humans. Our patient repeatedly tested negative for antibodies to HIV-1 and HIV-2 and for p24 antigen and had not kept any pets during the 2 years before her death. CD4 and CD8 lymphocyte counts were never determined.

From the onset of the disease, the patient had lymphopenia and a drastic increase in the number of band forms in her peripheral blood. These findings suggested a bacterial infection, but all cultures of the blood remained negative. The post-mortem examination revealed a striking lack of T- and B-lymphocytes in the spleen and severely suppressed granulopoiesis in the bone marrow, but a defined immune defect could not be diagnosed. When we retrospectively examined the lung, liver, and bone marrow biopsy specimens obtained in August 1994, we noted the absence of opportunistic pathogens during the first hospitalization of the patient.

It is most likely that our patient’s prolonged treatment with immunosuppressants, perhaps during an undiagnosed bacterial infection, provided an environment favorable for the development of systemic mycobacteriosis and P. carinii pneumonia. Although virtually nothing is known about the virulence of M. genavense, it is possible that in our patient the extensive histiocytic infiltration of the patient’s bone marrow contributed to her severe pancytopenia and thereby to the subsequent fatal complications.

We were unable to classify the underlying immunologic disorder or immune defect in our patient, but her family history suggests an inherited component. Ten years earlier, the patient’s younger sister presented at age 31 with an illness that was also characterized by high temperatures ($\leq40^\circ$C), arthralgias, and pericardial and pleural effusions. Neither septicemia nor a connective tissue disorder or a malignant disease could be proven. After treatment with high-dose prednisolone, she developed pancytopenia and had a fatal peritoneal hemorrhage. Autopsy revealed severe systemic mycobacteriosis that involved multiple thoracic and abdominal lymph nodes as well as the spleen. Postmortem cultures were reported to be positive for nontuberculous mycobacteria; no further effort to identify the species was made at that time.
A case of cervical lymphadenitis caused by a mycobacterium related, but not identical, to *M. genavense* was recently reported in a 41-year-old woman whose immune cells appeared functionally normal as far as was tested. It is of interest that an elder brother of this patient died at age 53 of a generalized infection with acid-fast bacteria that failed to grow in vitro [11]. Thus, both in this case and ours, an unidentified inherited immune deficiency might have contributed to the development of localized or systemic mycobacteriosis. This hypothesis is supported by two recent studies in which a lack of IL-12 production or a defective IFN-\(\gamma\) receptor was observed in several cases of familial disseminated nontuberculous mycobacteriosis [12, 13].

In conclusion, generalized infections with *M. genavense* are not restricted to HIV-infected patients but appear to occur in otherwise immunosuppressed persons as well. *M. genavense* infections should be considered in the differential diagnosis of mycobacteriosis whenever acid-fast bacteria are detected microscopically in specimens but fail to grow in vitro.

References