Recurrent, Multifocal *Mycobacterium avium-intercellulare* Infection in a Patient with Interferon-γ Autoantibody

N. Baerlecken, R. Jacobs, M. Stoll, R. E. Schmidt, and T. Witte

Clinic for Immunology and Rheumatology, Medical School of Hanover, Hanover, Germany

We describe a patient who experienced severe osteomyelitis because of *Mycobacterium avium-intercellulare* infection and high-titered autoantibodies against interferon-γ. In addition to antimycobacterial chemotherapy, we treated our patient with plasmapheresis and cyclophosphamide. The treatment induced a remission after 3 years of follow-up.

In Germany, the United Kingdom, the United States, and Japan, there have been reports concerning recurrent nontuberculous mycobacterial infection related to the presence of autoantibodies against interferon (IFN)–γ. Two of 12 described patients died, 7 have had no remission of illness after receiving long-term treatment with mycobacterial chemotherapy, and 3 seemed to recover slowly [1–5]. Most patients were treated with antimycobacterial chemotherapy alone or in combination with glucocorticoids and IFN-γ.

We treated a patient with a series of cyclophosphamide pulses and plasmapheresis and antimycobacterial chemotherapy. After 1.5 years, the symptoms of the infection were reduced significantly.

**Case report.** A 38-year-old woman, who had been born in the Philippines, was admitted to our hospital because of back pain, intermittent fever, and arthralgia in September 2004 (Figure 1). At this time, osteomyelitis in the spine was discovered by magnetic resonance imaging (MRI) and on the basis of an anti-nuclear antibody titer of 1:320 and epitheliod-cell granulomas in the liver with elevated liver enzyme levels. Malignancy was ruled out, epitheliod-cell granulomas were found in the lungs, and *Mycobacterium avium-intercellulare* was identified by polymerase chain reaction of sputum and bronchoalveolar lavage fluid specimens.

In March 2005, we identified *M. avium-intercellulare* in biopsy specimens of the liver, spine, and lung and in blood cultures. Antimycobacterial therapy was started, consisting of rifabutin, ethambutol, and azithromycin. Because of the patient’s severe chronic opportunistic infection, she was evaluated for immunodeficiency. However, the human immunodeficiency virus (HIV) test result was negative, the CD4 cell count was normal, no immunoglobulin classes or subclasses were reduced, and the T cell proliferation in response to CD3 antibodies, phytohemagglutinin, and concanavalin-A was normal. The patient’s medical history was notable for complete recovery from tuberculosis in 1992, when she lived in the Philippines. Therefore, an acquired immunodeficiency was suspected.

Indeed, during additional diagnostic procedures in September 2006, highly concentrated autoantibodies against IFN-γ were found. The binding of IFN-γ produced by peripheral blood mononuclear cells (PBMCs) from a healthy donor after ionomycin stimulation to beads carrying an IFN-γ antibody in the presence of the patient’s serum was tested using a multiplex assay (BD Cytometric Bead Array Human Th1/Th2 Cytokine Kit II; BD Biosciences). After mixing of cytokine capture beads and phycoerythrin-conjugated detection antibodies, we added the test samples to form sandwich complexes in different serum dilutions of 1:100 and 1:500. By flow cytometry we quantitatively measured interleukin (IL)–2, IL-4, IL-6, IL-10, tumor necrosis factor–α and IFN-γ protein levels in stimulated PBMCs in the presence of serum from a healthy donor or from the patient, with and without preclearing with A sepharose. The assay revealed a decreased level of IFN-γ binding to the beads in the presence of the patient’s serum and a normal level in the presence of the healthy donor’s serum (Figure 1). After IgG depletion of the patient’s serum by protein A sepharose absorption, the serum did not neutralize IFN-γ from the supernatant of stimulated PBMCs any more, demonstrating the presence of a neutralizing autoantibody against IFN-γ in the serum from the patient.

To analyze the blocking capacity of the patient’s serum on IFN-γ-mediated cellular functions, we performed the following experiment. Jurkat cells were incubated overnight in the presence or absence of IFN-γ (4 ng/mL). Fluorescent-activated cell sorting (FACS) analysis revealed a doubling of the mean fluorescence intensity of MHC-I surface expression in response to IFN-γ treatment, compared with the medium control. This
IFN-γ-induced increase in mean fluorescence intensity could be blocked by adding the patient’s serum in a dose-dependent manner. Nearly complete blockade of the MHC-I induction was achieved by a serum dilution of 1:10, 1:100, and 1:1000 but not by a serum dilution of 1:10,000. In contrast, pooled serum from healthy donors failed to block IFN-γ-induced MHC-I expression at any serum dilution. This experiment clearly shows that the patient’s serum contains anti–IFN-γ autoantibodies, which is able to inhibit IFN-γ-mediated biological effects in vitro.

Initial therapy involved plasmapheresis, cyclophosphamide pulses, and antimycobacterial therapy, and the concentration of circulating antibodies decreased to the extent that the patient’s immune system significantly improved. She therefore decided to discontinue antimycobacterial therapy in March 2007 without consulting the physicians. Cyclophosphamide therapy was continued, but disease activity increased. Only when quadruple antimycobacterial therapy was resumed could final remission be achieved. Computed tomography and MRI revealed no signs of activity of inflammation in the areas mentioned above. As of the most recent follow-up visit (after 1.5 years of treatment), the patient has had no symptoms of mycobacterial infection noted by blood cultures, and the C-reactive protein level has normalized (Figure 2). For the maintenance remission, she is now being treated with a triple antimycobacterial therapy regimen and low-dose prednisolone to control slight arthralgia.

**Discussion.** In the present report, we report a case of disseminated *M. avium-intercellulare* infection in the presence of IFN-γ autoantibodies. Chronic recurrent and multiple infections were so severe that we suspected an underlying immunodeficiency.

We excluded primary congenital and secondary immunodeficiency due to chronic infection with human immunodeficiency virus or Epstein-Barr virus and malignant and autoimmune diseases. Autoantibodies against IFN-γ seemed to be responsible for the patient’s rare, acquired immunodeficiency. IFN-γ is produced by Th1 and NK cells and is indispensable for the intracellular killing process and phagocytosis of intra-
cellular bacteria. Therefore, a loss of IFN-γ and a defect of the IFN-γ receptor in inherited disorders can cause a higher risk of infection with intracellular bacteria, such as mycobacteria. The pathomechanism that leads to formation of antibodies against IFN-γ and the reason for predominant affection of people from southeast Asia are unknown.

In reports by Kampmann et al [3] and Doffinger et al [1], substitution with recombinant IFN-γ and antimycobacterial therapy were combined. The patient described by Kampmann and colleagues showed slow improvement and became symptom free. However, the patient described by Doffinger and colleagues died of overwhelming *Mycobacterium chelonae* infection after 3 years of treatment with recombinant IFN-γ.

We initiated immunosuppressive therapy with plasmapheresis and cyclophosphamide in addition to antimycobacterial therapy to decrease the circulating autoantibodies against IFN-γ. Indeed, the concentration of autoantibodies against IFN-γ was reduced by immunosuppressive treatment, and clinical improvement was achieved. Rituximab could have been considered as an alternative therapy to cyclophosphamide. We chose to administer cyclophosphamide because it has been shown that it reduces autoantibody concentrations in many autoimmune disorders, whereas rituximab does not deplete plasma cells and does not necessarily reduce autoantibody concentrations. However, when the patient stopped antimycobacterial therapy without informing us, the immunosuppression alone was not efficient enough.

In conclusion, our patient, who had disseminated *M. avium-intercellulare* infection affected by autoantibodies against IFN-γ, recovered because of a combination of antimycobacterial and immunosuppressive therapy and has shown no sign of new infection with *M. avium-intercellulare* or other intracellular bacteria for >1.5 years. At least plasmapheresis and cyclophosphamide should be considered in future cases of acquired infection with atypical mycobacteria due to IFN-γ autoantibodies that are resistant to antimycobacterial therapy alone.

**Acknowledgments**

**Potential conflicts of interest.** All authors: no conflicts.

**References**


