Autoantibodies to Interferon-γ in a Patient with Selective Susceptibility to Mycobacterial Infection and Organ-Specific Autoimmunity

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We evaluated a patient with disseminated Mycobacterium tuberculosis and Mycobacterium chelonae infection, of which he died. He also developed autoimmune (type 1) diabetes and primary hypothyroidism. His serum contained a high titer of immunoglobulin G autoantibody to interferon-γ (IFN-γ) capable of blocking in vitro responses to this cytokine by peripheral blood mononuclear cells from normal donors. These results suggest that autoantibodies to IFN-γ can induce susceptibility to disseminated mycobacterial infection, which may be refractory to chemotherapy.

Increased susceptibility to mycobacteria of low-grade virulence (nontuberculous mycobacteria [NTM] and bacille Calmette-Guérin [BCG]) may be caused by genetic defects that impair IFN-γ– and IL-12–mediated immunity (Online Mendelian Inheritance in Man [OMIM] reference number 209950) [1, 2]. We describe, to our knowledge, for the first time, a patient with “acquired” deficiency of IFN-γ–mediated immunity caused by autoantibodies to this cytokine. This patient developed disseminated Mycobacterium tuberculosis and Mycobacterium chelonae infection, which resulted in his death, despite the administration of chemotherapy. He also had autoimmune polyendocrinopathy and recurrent oral candidiasis.

Case report. A 47-year-old, previously fit, Filipino man presented with fever, jaundice, ascites, and generalized lymphadenopathy in April 1995. Fully drug-sensitive M. tuberculosis was isolated from sputum samples and a lymph node specimen. Lymph nodes showed reactive hyperplasia but no granulomata. Liver biopsy revealed caseating granulomata and acid- and alcohol-fast bacilli. The patient was born to nonconsanguineous parents; his family had no significant medical history, and he had previously been vaccinated with BCG. He was treated with rifampin, isoniazid, pyrazinamide, and ethambutol. Rifampin therapy was stopped after 7 months; treatment with the other 3 drugs was continued for an additional 5 months. His sputum became sterile only after 6 months of chemotherapy, and complete clinical and microbiological recovery was achieved after 12 months.

Six months after recovery, in October 1996, the patient developed pericardial effusion, from which M. chelonae was cultured. Despite receiving treatment with isoniazid, pyrazinamide, ethambutol, amikacin, doxycycline, and clarithromycin, the disease progressed, with cutaneous, pleuropulmonary, lymph node, bone marrow, and hepatic involvement. M. chelonae was...
Table 2. Endocrine abnormalities developed by a patient with selective susceptibility to mycobacterial infection and organ-specific autoimmunity.

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<tr>
<th>Endocrine disease</th>
<th>Biochemical evidence</th>
<th>Autoimmune serological findings</th>
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<tbody>
<tr>
<td>Type I diabetes</td>
<td>Hyperglycemia, ketosis, weight loss</td>
<td>Islet cell antibodies (weak positive), anti-GAD</td>
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<tr>
<td>Hypogonadism</td>
<td>Testosterone level of &lt;0.7 nmol/L (normal range, 9–41 nmol/L); FSH level, 18.6 U/L (normal range, 1–10 U/L); sex hormone–binding globulin level was normal</td>
<td>Negative for anti–adrenal antibodies; negative for anti–testicular antibodies</td>
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<tr>
<td>Hypothyroidism</td>
<td>T4 level, 10 pmol/L (normal range, 11–25 pmol/L); TSH level, &gt;50 MU/L (normal range, 0.3–4.0 MU/L)</td>
<td>Negative for anti–thyroid peroxidase antibodies</td>
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**NOTE.** FSH, follicle-stimulating hormone; GAD, glutamic acid decarboxylase; TSH, thyroid-stimulating hormone.

cultured from several sites, including the pericardium and bone marrow.

The patient had normal or elevated blood lymphocyte subpopulations and polyclonally elevated serum immunoglobulin levels (data not shown). HIV and human T lymphotropic virus type 1 antibodies were not detected. His PBMCs showed a normal proliferative response to purified-protein derivative (PPD), but there was defective IFN-γ secretion in vitro to PPD and phytohaemagglutinin (PHA) in the presence of 5% autologous serum; this could not be restored by the addition of IL-12. IL-12 secretion in response to lipopolysaccharide (LPS) was also impaired (table 1).

IFN-γ therapy (90 µg given subcutaneously 3 times per week) was started in April 1998. Subsequently, there was resolution of the cutaneous disease and sustained improvement elsewhere, although M. chelonae continued to be isolated from samples of sputum and fluid draining from a pericardiocutaneous fistula. In July 1998, the patient developed sclerosing cholangitis. Cytomegalovirus (CMV) DNA was detected in blood samples by PCR. The patient was treated with ganciclovir, and a biliary stent was inserted. Eighteen months later, he developed significant hypercalcaemia requiring corticosteroid treatment. The frequency of IFN-γ administration was reduced to twice per week. He also developed recurrent oral candidiasis in the last 2 years of his life.

In April 1999, 1 year after the patient commenced IFN-γ treatment, he developed insulin-dependent diabetes mellitus. After 2 years, in April 2000, he had developed primary hypothyroidism and hypogonadism (table 2).

Three years after the patient started IFN-γ therapy, when he
was also receiving prednisolone to control hypercalcemia, his mycobacterial infections worsened, and he developed an *M. chelonae* psoas abscess. He subsequently died of overwhelming mycobacterial infection.

**Material and methods.** All patient and control samples were obtained after informed consent was provided. PBMCs were obtained and cultivated, as described elsewhere [3]. ELISA for IFN-γ, TNF-α (CLB), and IL-12 (R&D Systems) were performed in accordance with the manufacturer’s instructions. Whole-blood assays were performed, as described elsewhere [4], PHA and LPS were provided by Sigma; PPD was provided by Evans. For the detection of anticytokine antibodies, we coated ELISA plates (Nunc Maxisorp) with recombinant human IFN-γ (2 μg/mL; ImmuKin), TNF-α, or IL-12 and blocked with 5% skimmed milk. Plates were washed and incubated with the serum. After further washing, the plate was incubated with either of the following peroxidase-labelled conjugates: rabbit anti-human IgG or IgG/IgM/IgA (Dako). Revelation was done using tetramethyl-3,5,3′,5′-benzidine (Sigma) as substrate.

Human recombinant IFN-γ or bovine serum albumin (BSA) were coupled to a solid support using the AminoLink Plus Immobilization Kit (Pierce), in accordance with the manufacturer’s instructions. Serum-binding and elution were performed in accordance with the manufacturer’s instructions. Eluates were dialyzed against RPMI 1640.

**Results.** The development of disseminated mycobacterial infections and the initial investigation of cytokine production suggested a possible defect in the IL-12–dependent INF-γ pathway. Known Mendelian defects in this pathway were excluded by sequencing the IFNGRI, IFNGR2, STAT1, IL12B, and IL-12RB1 genes [2].

Because this patient also developed organ-specific autoimmune disease, we considered the possibility that his susceptibility to mycobacterial infection could be associated with inhibitory autoantibodies to key cytokines and receptors involved in antimycobacterial immunity. We found a high titer of IgG antibodies to IFN-γ in the patient’s serum sample (figure 1); in contrast, the levels of anti–IFN-γ antibody in serum samples obtained from 60 patients with bacteriologically proven tuberculosis, from 15 patients with unusual NTM infection, and from 57 healthy control subjects were undetectable or there was a low titer (figure 2). Autoantibodies to IL-12p70 or TNF-α were not detected in our patient (data not shown).

Whole-blood preparations derived from healthy donors were activated with LPS, with and without INF-γ, for 48 h, and TNF-α production was measured in the presence of the patient’s serum. Results summarized in figure 3 show that the patient’s serum inhibited the IFN-γ–dependent augmentation of LPS-induced TNF-α production by PBMCs in a concentration-dependent manner.

The patient’s serum was passed either through an affinity column coupled to IFN-γ or a BSA-coupled control column. The passage through an IFN-γ affinity column, but not through a BSA column, removed the ability of the patient’s serum to inhibit the biological activity of IFN-γ (figure 3). The eluted fractions from the IFN-γ–coupled column, but not from the BSA-coupled column, contained IgG antibodies specific for IFN-γ (as determined by ELISA; data not shown) and were able to inhibit the biological activity of IFN-γ in vitro (figure 3).

**Discussion.** This report describes a 47-year-old, previously healthy Filipino man who developed disseminated infection with *M. tuberculosis* and *M. chelonae*. He developed autoimmune (type 1) diabetes, primary hypothyroidism, and hypogonadism, as well as recurrent candidiasis. The patient died of overwhelming mycobacterial infection.

IgG autoantibodies to INF-γ were detected in serum samples obtained from the patient, including samples obtained before IFN-γ therapy. These antibodies abrogated INF-γ–mediated in vitro TNF-α production. The serum antibody titers are extremely high (up to 10^9). Considering that patient weighed 70 kg and had a vascular volume of 5 L, our in vitro inhibition data (figure 3) suggest that the patient’s anti–IFN-γ titer could account for a significant inactivation of endogenous INF-γ in vitro.
Figure 3. Patient’s serum inhibiting INF-γ–induced production of TNF-α in vitro. The inhibitory activity can be removed by affinity chromatography through an IFN-γ–coupled column. Whole blood samples obtained from healthy control subjects were incubated at a final dilution of 1:5 in RPMI 1640 and were activated by adding lipopolysaccharide (LPS), with or without recombinant human IFN-γ. The patient’s serum was capable of inhibiting the enhancement of TNF-α production induced by INF-γ (H18549). Basal TNF-α production induced by LPS was not affected (data not shown). The patient’s serum was passed over either an IFN-γ–coupled affinity column or a bovine serum albumin (BSA)–coupled control column. The ability of the serum, before and after passage and of the eluted fractions, to suppress IFN-γ–driven enhancement of TNF-α production was determined. The BSA column is not able to remove any inhibitory activity from the serum; in contrast, the serum has lost its inhibitory activity after the passage through the IFN-γ–coupled affinity column (H18554). The ability to suppress IFN-γ–driven TNF-α production is detectable in the eluate of the IFN-γ column but not of the BSA column (O). The reduced ability of the eluates to suppress the biological activity of IFN-γ, compared with that of the original serum (pre), is due to dilution during the elution process (5×) and, in the case of the eluate from the IFN-γ column, also to an incomplete recovery of the bound antibodies. Data are means of triplicates ± SD. One representative experiment out of 3 is shown.

Observations in humans with a deficient IL-12–mediated IFN-γ pathway have shown that IFN-γ is essential for the control of poorly pathogenic mycobacterial infection [1]. Therefore, it is highly probable that this patient’s susceptibility to disseminated mycobacterial infection was due to autoantibody-mediated inhibition of IFN-γ function. Because the patient lived in The Philippines for many years, it is likely that his tuberculosis occurred after primary infection and was due to reactivation or reinfection. Systemic spread of tuberculosis is unusual in adults with postprimary tuberculosis. The clinical response to antituberculous therapy was slow despite administration of antibiotic susceptibility–guided treatment and good patient compliance. Therefore, it is plausible that the antibodies to IFN-γ contributed to the patient’s susceptibility to infection with M. tuberculosis, as well as the failure to prevent its dissemination. The patient developed sclerosing cholangitis, which was probably due to disseminating mycobacterial disease, with CMV as a possible contributor. Viral infections with herpes viruses have been described in patients with IFN-γ receptor deficiencies [6], which suggests that the patient’s anti–IFN-γ antibody might have caused or contributed to the reactivation of CMV.

“Natural” anti–INF-γ antibodies have been reported in patients with viral diseases and tuberculosis and in healthy subjects, but the potential pathogenic impact of these antibodies has not been established [7–9]. There was only weak or no anti–IFN-γ activity in our 3 control groups (figure 2).

The patient also developed chronic candidiasis and multiple endocrinopathies similar to autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (OMIM reference number 240300) [10], which is caused by mutations in the autoimmune regulator (AIRE) [11, 12]. Sequencing of AIRE in our patient revealed no pathogenic mutation.

Our data strongly suggest that neutralizing autoantibodies to IFN-γ resulted in susceptibility to treatment-refractory mycobacterial infection. Although this appears to be a rare condition, we suggest that individuals with disseminated tuber-
culous or late-onset nontuberculous mycobacterial infection should be investigated for autoantibodies to IFN-γ. The concomitant presence of autoimmune endocrinopathies may alert the clinician to consider this differential diagnosis. If autoantibodies to IFN-γ were detected, plasmapheresis, high-dose intravenous immunoglobulin, or rituximab would be options for treatment.

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References


### Figure 1

Patient’s serum inhibiting IFN-γ–induced production of TNF-α in vitro. The inhibitory activity can be removed by affinity chromatography through an IFN-γ–coupled column. Whole blood samples obtained from healthy control subjects were incubated at a final dilution of 1:5 in RPMI 1640 and were activated by adding lipopolysaccharide (LPS), with or without recombinant human IFN-γ. The patient’s serum was capable of inhibiting the enhancement of TNF-α production induced by IFN-γ (■). Basal TNF-α production induced by LPS was not affected (data not shown). The patient’s serum was passed over either an IFN-γ–coupled affinity column or a bovine serum albumin (BSA)–coupled control column. The ability of the serum, before and after passage and of the eluted fractions, to suppress IFN-γ–driven enhancement of TNF-α production was determined. The BSA column is not able to remove any inhibitory activity from the serum; in contrast, the serum has lost its inhibitory activity after the passage through the IFN-γ–coupled affinity column (□). The ability to suppress IFN-γ–driven TNF-α production is detectable in the eluate of the IFN-γ column but not of the BSA column (○). The reduced ability of the eluates to suppress the biological activity of IFN-γ, compared with that of the original serum (pre), is due to dilution during the elution process (5×) and, in the case of the eluate from the IFN-γ column, also to an incomplete recovery of the bound antibodies. Data are means of triplicates ± SD. One representative experiment out of 3 is shown.