Disseminated *Mycobacterium genavense* infection after immunosuppressive therapy shows underlying new composite heterozygous mutations of β1 subunit of IL-12 receptor gene

To the Editor:

We describe a 35-year-old white Italian female patient with abdominal lymphadenopathy and diarrhea who had received immunosuppressive therapy with corticosteroids and azathioprine since the age of 23 because of type I autoimmune chronic hepatitis associated with α1-antitrypsin deficiency (genotype MZ). This diagnosis was based on positivity to autoantibodies and liver biopsy that showed portal and periportal lymphoplasmacytic infiltrates with bridging necrosis. In the same liver biopsy periodic acid-Schiff diastase staining showed diastase-resistant pink globules associated with immunoreactivity for α1-antitrypsin.

At the age of 32, the patient presented with high fever with hepatic, mesenteric, and retroperitoneal lymphadenopathy. At the same time, while she was already off azathioprine treatment, the therapy with corticosteroids was also stopped. In the next months she developed diarrhea and weight loss that led us to hypothesize she had a malabsorption syndrome. Biopsies of bone marrow and lymph nodes showed the presence of macrophages containing acid-fast bacilli; liver biopsy showed the presence of granulomatous hepatitis positive for Ziehl-Neelsen stain in association with the previously described pathologic findings (piecemeal and occasionally bridging necrosis with portitis), indicative of chronic active hepatitis. Detection of *Mycobacterium genavense*–specific DNA was achieved in multiple hepatic biopsies that were embedded in paraffin by genetic amplification (culture in enriched liquid medium was persistently negative). The patient was not vaccinated for BCG and reported no previous history of immunodeficiency disorders or chronic diseases in her family.

Endoscopic duodenal biopsies showed macrophage infiltration of intestinal mucosa (Fig 1, *A*), as shown by CD68 staining (Fig 1, *D*) that could resemble other granulomatous diseases of the intestine such as chronic granulomatous disease or Whipple disease. However, neutrophil respiratory burst was normal and periodic acid-Schiff staining was negative, while acid-fast microorganisms could be identified in macrophages (Fig 1, *B* and *C*). Therefore, antimycobacterial therapy to target *M genavense* infection was started in February 2010 with amikacin,

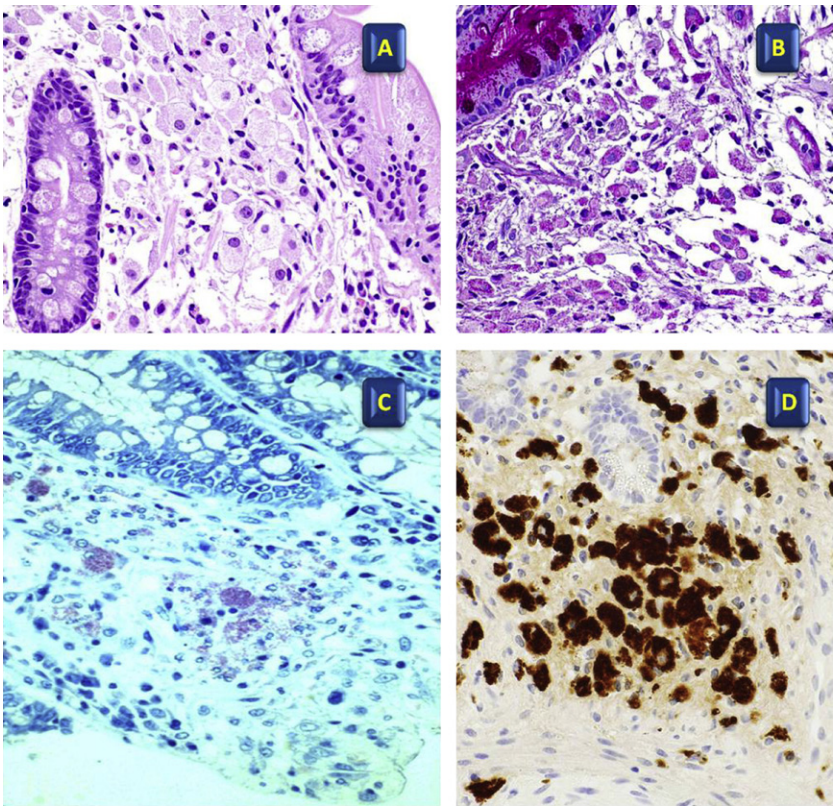

FIG 1. Histologic and immunologic characterization of a patient with *Mycobacterium genavense* infection. Sections of duodenal biopsies were obtained from a patient with *M genavense* infection **(A-D)** and stained for hematoxylin and eosin **(A)**, periodic acid-Schiff **(B)**, Ziehl-Neelsen **(C)**, and CD68 (*brown*, **D**). Biopsies from the patient showed a moderate dermal mononuclear infiltrate in lamina propria **(A)**, constituted by $CD68^+$ macrophages **(D)** and containing Ziehl-Neelsen–positive bodies **(C)**, whereas periodic acid-Schiff stain was negative **(B)**. Magnification: ×40 (*A* and *D*), ×20 (*B* and *C*).

moxifloxacin, clarithromycin, and rifabutin. The clinical status was characterized by persistent diarrhea, hypoalbuminemia (2.5 g/dL), hypogammaglobulinemia (IgG, 504 mg/dL; IgA, 165 mg/dL; IgM, 29 mg/dL), and ascites. Small bowel and capsule endoscopy showed typical features of intestinal lymphangiectasia that was probably a consequence of the abdominal lymphadenopathy. Evaluation of the immune system showed lymphopenia (810 cells/μL), impaired thymic output that was based on the number of recent thymic emigrants ($CD3^+CD4^+CD45RA^+CD31^+$, 3.6%), decreased levels of T-cell receptor excision circles (185 cells/mL), and abnormal distribution of lymphocyte subsets ($CD3^+$, 145 cells/μL; $CD4^+$, 96 cells/μL; $CD8^+$, 45 cells/μL; $CD19^+$, 38 cells/μL; $CD16^+$, 231 cells/μL), whereas lymphoproliferative response to mitogens was normal. In the hypothesis of Mendelian susceptibility to mycobacterial diseases (MSMD),[1-4] we have evaluated the biological response of lymphocytes and of PBMCs to, respectively, IL-12 or IFN-γ. Analysis of IFN-γ secretion showed a profound defect of IFN-γ production in response to increasing concentrations of IL-12 (50, 200, 500 ng/mL) in the cell cultures from the patient, whereas cells from the healthy control subject showed a normal release of IFN-γ (Fig 2, *A*). Conversely, a normal secretion of TNF-α in response to IFN-γ and LPS was detected in PBMCs from the patient as seen in cells from the healthy control subject (data not shown).

Analysis of cell surface expression of β1 subunit of IL-12 receptor (IL-12Rβ1) on PHA-activated T lymphocytes from both the patient and the healthy control subject with the use of 2 distinct mAbs directed against the human anti–IL-12Rβ1 (mAbs 69310 and 2.4E6) showed that the cells from the patient completely lacked IL-12Rβ1 expression (Fig 2, *B*). In addition, analysis of signal transducer and activator of transcription 4 (STAT4) phosphorylation after stimulation with IL-12 or IFN-α by flow cytometry showed an impaired phosphorylation of STAT4 after treatment with IL-12 but a normal response to IFN-α, whereas control cells displayed normal phosphorylation to both cytokines (Fig 2, *C*).

Next, we sequenced the 17 coding exons and the flanking intronic regions of *IL12RB1* in the genomic DNA extracted from EDTA-treated whole blood samples of the patient and of both parents. Molecular analysis of the *IL12RB1* gene in the patient showed a heterozygous nonsense nucleotide substitution (C>T) at position 634, inherited from the father, leading to a premature stop codon in the exon 7 (p.Arg212X), and a heterozygous nucleotide substitution (G>A) at the first intronic nucleotide after exon 11 (position 1327+1) that affected the donor splicing site, which was inherited from the mother (Fig 1, *D*). Sequencing of the *IL12RB1* cDNA coding region with primers spanning from exon 9 to exon 12 showed an in-frame deletion of exon 11. Both mutations were never reported in patients with MSMD.

At the time of writing the patient has completed 27 months of therapy with clarithromycin, moxifloxacin, and rifabutin,

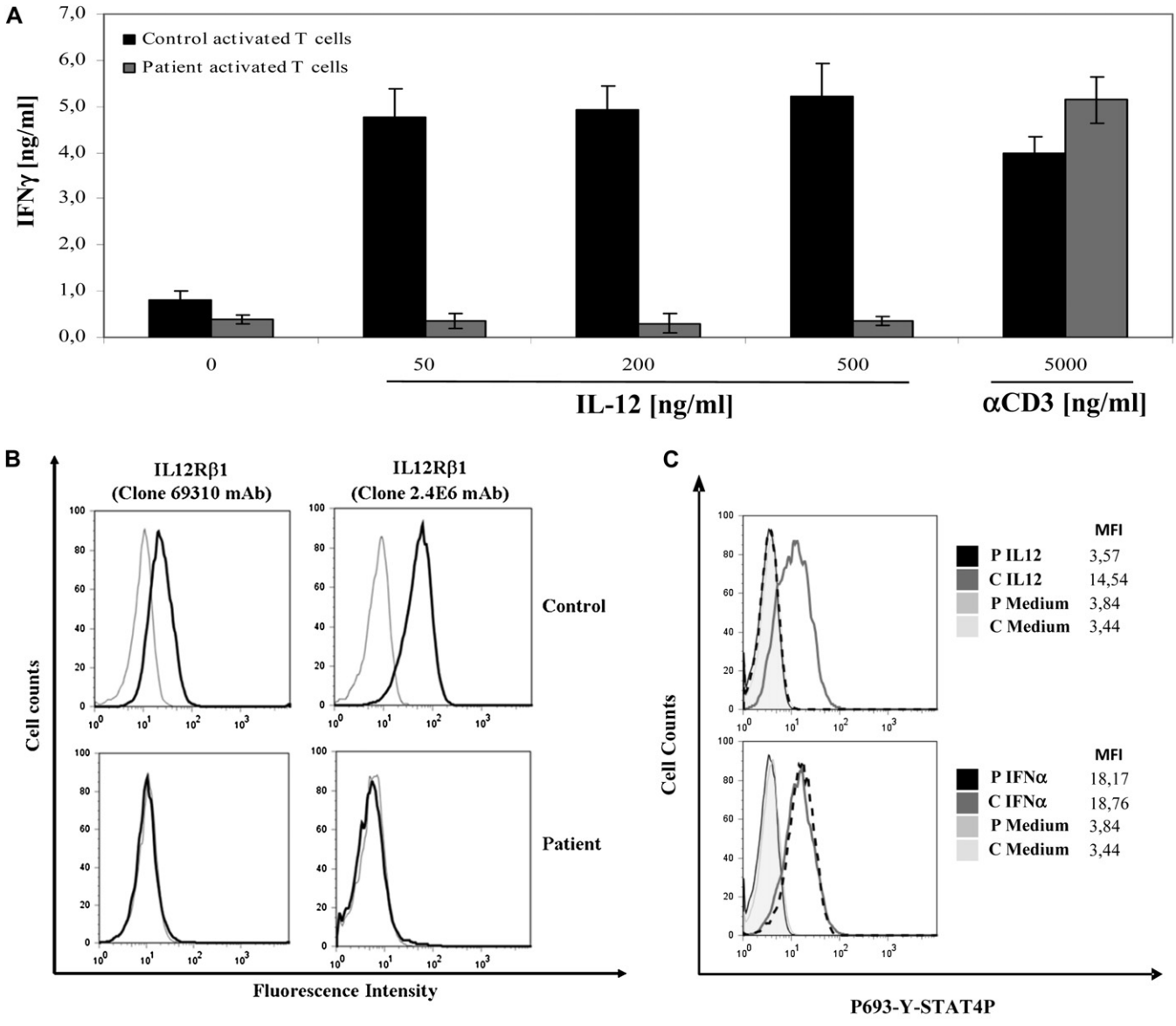

FIG 2. Abnormal response to Il-12 is associated to composite heterozygous mutations of IL12RB1. **A,** PHA-activated T lymphocytes from the patient *(gray bar)* or a healthy control subject *(black bar)* were

associated with IFN-γ therapy at doses up to 200 μg/m^2; she shows slow clinical improvement characterized by weight gain and absence of fever and diarrhea.

We have described a case of composite heterozygous mutations of *IL12RB1* gene encoding for the β1 subunit of IL-12 receptor that is associated with disseminated *M genavense* infection. In a recent survey of 141 patients with IL-12Rβ1 deficiency, isolated BCG infection has been observed in most patients (43 subjects of 102 index cases), environmental mycobacteria were observed in 6 patients, and the remaining patients had infections caused by *Mycobacterium tuberculosis* (2 patients), other intracellular pathogens (mostly *Salmonella*, but also *Klebsiella* and *Nocardia* species), or combinations of the above-described pathogens.[5,6] Infection by *M genavense* was reported in a single patient with IL-12Rβ1 deficit, suggesting that this pathogen is a rare cause of MSMD. In addition, IL-12Rβ1 deficiency has incomplete clinical penetrance because 8 of 29 known genetically affected siblings investigated in the survey did not develop MSMD-related infections.[5] This observation suggests that environmental factors, including the route of infection (eg, subcutaneous injection of BCG), or the use of immunosuppressant drugs can facilitate the development of disseminated infections as observed in our patient who was receiving an immunosuppressive drug for the treatment of autoimmune hepatitis. Likewise, *M genavense* infection was reported in patients receiving immunosuppressive therapy after transplantation[7] or in patients with AIDS.[8,9] Development of lymphopenia is not usually observed in patients with IL-12Rβ1 deficiency, although the patient that we describe has shown a profound lymphopenia that might be related to a defect of lymphocyte generation in bone marrow or to intestinal lymphangiectasia.

In conclusion, our observation suggests that development of disseminated mycobacterial infection in patients receiving immunosuppressive treatment could constitute a presenting phenotype of MSMD.

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stimulated with IL-12 (50, 200, or 500 ng/mL) or human anti-CD3 mAb 5 μg/mL or left unstimulated (medium). The supernatant fluids were collected after 96 hours and analyzed for the production of IFN-γ by FlowCytomix Assay. **B,** PHA-activated T lymphocytes from the patient *(lower histograms)* or a healthy control subject *(upper histograms)* were stained with anti-human IL12Rβ1 (clone 69310) mAb *(left histograms, thick line)* or anti-human IL12Rβ1 (clone 2.4E6) mAb *(right histograms, thick line)* or with matched isotype control *(thin line)*, directly conjugated to phycoerythrin. **C,** STAT4 phosphorylation. Flow cytometric analysis of STAT4 phosphorylation of PHA-activated T lymphocytes after treatment with IL-12 (200 ng/mL; *upper panel*), IFN-α (10 U/μL; *lower panel*), or medium alone for 20 minutes at 37°C. Mean fluorescence intensity (MFI) is indicated. P, patient; C, control. **D,** Schematic representation of the 17 exons of wild-type and patient IL12Rβ1 mRNA. Coding exons are indicated with Roman numbers and delineated by vertical bars; exon 1 contains peptide leader sequence (L), and exon 14 contains the transmembrane domain.