

Mutation in the signal-transducing chain of the interferon-gamma receptor and susceptibility to mycobacterial infection.

S E Dorman, S M Holland

J Clin Invest. 1998;101(11):2364-2369. <https://doi.org/10.1172/JCI2901>.

Research Article

IFN-gamma is critical in the immune response to mycobacterial infections, and deficits in IFN-gamma production and response have been associated with disseminated nontuberculous mycobacterial infections. Mutations in the IFN-gamma receptor ligand-binding chain (IFNgammaR1) have been shown to confer susceptibility to severe infection with nontuberculous mycobacteria. However, mutations in the IFN-gamma receptor signal-transducing chain (IFNgammaR2) have not been described. We describe a child with disseminated *Mycobacterium fortuitum* and *M. avium* complex infections and absent IFN-gamma signaling due to a mutation in the extracellular domain of IFNgammaR2. In vitro cytokine production by patient PBMCs showed 75% less PHA-induced IFN-gamma production than in normal cells, while patient PHA-induced TNF-alpha production was normal. The normal augmentation of TNF-alpha production when IFN-gamma was added to endotoxin was absent from patient cells. Expression of IFNgammaR1 was normal, but there was no phosphorylation of Stat1 in response to IFN-gamma stimulation. DNA sequence analysis of the gene for IFNgammaR2 showed a homozygous dinucleotide deletion at nucleotides 278 and 279, resulting in a premature stop codon in the protein extracellular domain. This novel gene defect associated with disseminated nontuberculous mycobacterial infection emphasizes the critical role that IFN-gamma plays in host defense against mycobacteria.

Find the latest version:

<https://jci.me/2901/pdf>



Mutation in the Signal-transducing Chain of the Interferon- γ Receptor and Susceptibility to Mycobacterial Infection

Susan E. Dorman and Steven M. Holland

Laboratory of Host Defenses, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892-1886

Abstract

IFN- γ is critical in the immune response to mycobacterial infections, and deficits in IFN- γ production and response have been associated with disseminated nontuberculous mycobacterial infections. Mutations in the IFN- γ receptor ligand-binding chain (IFN γ R1) have been shown to confer susceptibility to severe infection with nontuberculous mycobacteria. However, mutations in the IFN- γ receptor signal-transducing chain (IFN γ R2) have not been described. We describe a child with disseminated *Mycobacterium fortuitum* and *M. avium* complex infections and absent IFN- γ signaling due to a mutation in the extracellular domain of IFN γ R2. In vitro cytokine production by patient PBMCs showed 75% less PHA-induced IFN- γ production than in normal cells, while patient PHA-induced TNF- α production was normal. The normal augmentation of TNF- α production when IFN- γ was added to endotoxin was absent from patient cells. Expression of IFN γ R1 was normal, but there was no phosphorylation of Stat1 in response to IFN- γ stimulation. DNA sequence analysis of the gene for IFN γ R2 showed a homozygous dinucleotide deletion at nucleotides 278 and 279, resulting in a premature stop codon in the protein extracellular domain. This novel gene defect associated with disseminated nontuberculous mycobacterial infection emphasizes the critical role that IFN- γ plays in host defense against mycobacteria. (J. Clin. Invest. 1998. 101:2364–2369.) Key words: IFN type II • IFN receptors • atypical mycobacterium infections • TNF- α • signal transduction

Introduction

Human and animal data have clearly identified IFN- γ as critical in the immune response to mycobacteria. Defects in this pathway have been associated with severe mycobacterial infections in children. Complete deficiency of the IFN- γ receptor ligand-binding chain, designated IFN γ R1,¹ has been associated with inherited susceptibility to infection with nontuberculous

mycobacteria, including BCG (1–3). These mutations resulted in failure to detect IFN γ R1 on the cell surface, and reduced monocyte response to the addition of IFN- γ in vitro. We describe a patient with disseminated nontuberculous mycobacterial infection and normal surface display of IFN γ R1. His cells were unable to respond to addition of IFN- γ in vitro due to a mutation in the IFN- γ receptor signal-transducing chain, IFN γ R2. This novel gene defect associated with disseminated nontuberculous mycobacterial infection emphasizes the critical role that IFN- γ plays in host defense against mycobacterial infection.

Methods

Case report

A male was born at 26 wk gestation without obvious developmental abnormalities. As an infant he had two episodes of otitis media and one episode of thrush, all of which responded promptly to standard therapies. He received prescribed childhood immunizations; he did not receive BCG. At 20 mo old, the child developed a cough with pulmonary infiltrates that did not resolve despite clarithromycin and trimethoprim/sulfamethoxazole. At 2 yr old, he developed lymphadenopathy, hepatosplenomegaly, and fevers. Biopsy of an axillary lymph node showed capsular fibrosis and histiocytic infiltration without abscesses or granulomata. Acid-fast bacilli were present on staining, and cultures grew *Mycobacterium fortuitum* and *M. avium* complex. Therapy with rifabutin, azithromycin, ciprofloxacin, ethambutol, and amikacin was begun resulting in modest improvement in fever, activity level, and weight gain. 2 mo later, adjuvant therapy with subcutaneous IFN- γ was added without appreciable clinical response. The discontinuation of amikacin led to progressive worsening of generalized lymphadenopathy. At the time of writing, the patient continues to have persistent infection, despite continued intensive antibiotic therapy. Despite his infections, he has continued to grow along the 25th percentile for age.

The patient's mother was of English descent, and his father was of English and Portuguese descent; they were not known to be related. Both parents and an older male sibling were healthy. A maternal aunt had reportedly been diagnosed with tuberculosis at age 3 yr, and subsequently developed cervical lymphadenopathy; she died at the age of 26 of chronic aggressive hepatitis.

In the patient, laboratory studies at the time of diagnosis of mycobacterial infection (prior to IFN- γ therapy) showed mild normocytic anemia, with a white blood count (WBC) 41,000 cells/mm³, with 9% lymphocytes (3,700 cells/mm³), and a mildly reduced CD4 $^{+}$ T lymphocyte subset (1,155 cells/mm³, 31%). Quantitative immunoglobulins were normal, as was the reduction of nitroblue tetrazolium by polymorphonuclear leukocytes. Tests for HIV by ELISA and PCR methods were negative. Serologic testing for tetanus antitoxoid antibody showed a normal post vaccination level.

Subjects. Written informed consent was obtained for all subjects according to National Institutes of Allergy and Infectious Diseases protocol 93-I-0119.

In vitro PBMC cytokine production. We prepared PBMCs from heparinized whole blood, isolated by centrifugation through lymphocyte-separation gradient (BioWhittaker, Inc., Walkersville, MD), then washed with HBSS. 10⁶/ml PBMC were plated in 1 ml of complete

Address correspondence to Steven M. Holland, M.D., National Institutes of Health, Building 10, Room 11N103, 10 Center Drive, MSC 1886, Bethesda, MD 20892-1886. Phone: 301-402-7684; FAX: 301-402-4369; E-mail: smh@nih.gov

Received for publication 23 January 1998 and accepted in revised form 23 March 1998.

1. Abbreviation used in this paper: IFN γ R1, IFN- γ receptor ligand-binding chain.

RPMI (RPMI 1640, 2 mM glutamine, 20 mM Hepes, 0.01 mg/ml gentamicin) with 10% FCS onto 24-well plates. We stimulated selected wells with 1% PHA (Life Technologies, Gaithersburg, MD), 200 ng/ml *Escherichia coli*-derived LPS (Sigma Chemical Co., St. Louis, MO), LPS plus 1,000 U/ml IFN- γ (Genentech, Inc., South San Francisco, CA), PHA plus 1 ng/ml IL-12 heterodimer (IL-12; R&D Systems, Minneapolis, MN) or PHA plus 1 μ g/ml anti-IL-12 antibody (R&D Systems). PBMC were stimulated for 48 h at 37°C and then supernatants frozen at -20°C for cytokine determination. We thawed the samples and examined them for IFN- γ and TNF- α concentrations in duplicate using commercial ELISA kits (R&D Systems) as specified by the manufacturer.

We performed proliferation of PBMC in response to 1% PHA over 48 h in triplicate in flat-bottom 96-well plates in 200 μ l at a concentration of 10⁶ PBMC/ml, with an 8-h [³H]thymidine pulse at the end of the incubation.

Fluorescence-activated cell sorting. We performed flow cytometry using a FACScan flow cytometer (Becton Dickinson & Co., San Jose, CA). We determined the monocyte gate using anti-CD14 antibodies (PharMingen, San Diego, CA); we subsequently identified monocytes with forward and side scatter. We used anti-IFN γ R1 antibody (catalog #1224-00, Genzyme Corp., Cambridge, MA) and anti-IFN γ R2 antibody (catalog #80-4257-01, Genzyme Corp.) unconjugated with a goat anti-mouse antibody labeled with FITC (Caltag, San Francisco, CA). Anti-CD3 (Becton Dickinson), anti-CD4 (Coulter Immunotech, Westbrook, ME), anti-CD16/56, and anti-CD20 (both obtained from Becton Dickinson) were directly conjugated.

Sequence analysis of DNA for the genes for IFN γ R1 and IFN γ R2. For sequence analysis of complementary DNA (cDNA) for the genes for IFN γ R1 and IFN γ R2, total RNA was extracted from PBMC in RNAzol B (Tel-Test, Friendswood, TX). Complementary DNA was synthesized using SuperScript II RNase H-reverse transcriptase (Life Technologies) according to the manufacturer's instructions. The open reading frame of the IFN γ R1 gene was amplified by PCR with primers 5'GTTGGAGGCCAGCGACCGTCGGTA3' and 5'CCAGGAAAAATCAGACTTCAAAG3' designed from published sequence data (4). The open reading frame of the IFN γ R2 gene was amplified by PCR with primers 5'ACCTGAGCCG-CCGCCGAGCG3' and 5'TCCGATGGCTTGATCTCTC3' designed from published sequence data (5). Genomic DNA was extracted from polymorphonuclear leukocytes. Exon III and portions of the flanking introns of IFN γ R2 were amplified by PCR with primers 5'CTGCAGGAATTCTGTGAATTG3' and 5'CTCTTACCATTC-CGATAGTG3' designed from published sequences (5, 6) and from an intronic sequence kindly provided by Dr. Sidney Pestka (Robert Wood Johnson Medical School, Piscataway, NJ). We purified PCR products with the Wizard PCR Prep kit (Promega Inc., Madison, WI) according to the manufacturer's instructions, and then sequenced bidirectionally with the Sequenase CyclistTM Taq DNA Sequencing kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions.

Western blotting for Stat1. We maintained EBV-transformed B cells from the patient and a normal subject in complete RPMI with 20% FCS. For 48 h before stimulation, the cells were incubated in complete RPMI with 5% FCS and then incubated for 2 h in serum-free media. 1 \times 10⁷ cells were stimulated with 1,000 U/ml IFN- γ or an equivalent volume of sterile water for 15 min at 37°C and then solubilized with 0.5 ml of lysis buffer as described (7). After centrifugation to remove insoluble components, the supernatants were boiled for 8 min in an equal volume of SDS sample buffer. A 25- μ l aliquot of each sample was resolved on an 8% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane using the Xcell II Blot System (Novex, San Diego, CA) according to the manufacturer's protocol. The membrane was blocked overnight at 4°C using 5% nonfat dry milk in phosphate buffered saline plus 0.1% Tween-20 (PBST), and probed with polyclonal rabbit antibody directed against phosphorylated Stat1 (New England Biolabs, Beverly, MA) for 4 h at room temperature. The membrane was washed in PBST, incubated

for 1 h with horseradish peroxidase (HRP)-conjugated donkey anti-rabbit antibody (Amersham Corp., Buckinghamshire, England), and developed by the enhanced chemiluminescence (ECL) method (Amersham Corp.) according to the manufacturer's instructions. The membrane was stripped and reprobed with monoclonal mouse anti-Stat1 cytoplasmic terminus (Transduction Laboratories, Lexington, KY) for 1 h at room temperature, washed, incubated for 1 h with HRP-conjugated sheep anti-mouse antibody (Amersham Corp.), and developed with the ECL system. Lysate prepared from a human epidermoid carcinoma cell line (Transduction Laboratories) was used as a positive control for phosphorylated Stat1.

Results

We sought to determine whether there were any abnormalities in cytokine production or lymphocyte proliferation in this patient's cells. Proliferation of patient PBMC in response to PHA was normal, indicating that monocyte signaling for lymphocyte proliferation and lymphocyte proliferation were intact. However, as Fig. 1 shows after stimulation with PHA, the patient's cells produced low amounts of IFN- γ compared with cells from his parents and normal controls (patient 1,984 \pm 1,681 pg/ml, normal subjects 8,125 \pm 765 pg/ml). The addition of IL-12 to PHA in the PBMC cultures caused no appreciable upregulation of IFN- γ production from normal controls or the patient's parents (Fig. 1). In contrast, patient cells showed a dramatic increase in IFN- γ production with the addition of IL-12 (patient, 5.58-fold increase, normal controls, 1.04-fold increase). The addition of blocking antibodies to IL-12 led to a 30% decline in IFN- γ production in normals, compared with a 14% decline in the patient (data not shown). These data indicated that the patient had an intact IL-12 response but was producing low amounts of endogenous IL-12.

Production of TNF- α by patient PBMC varied depending on the stimulus used. In response to PHA, patient cells pro-

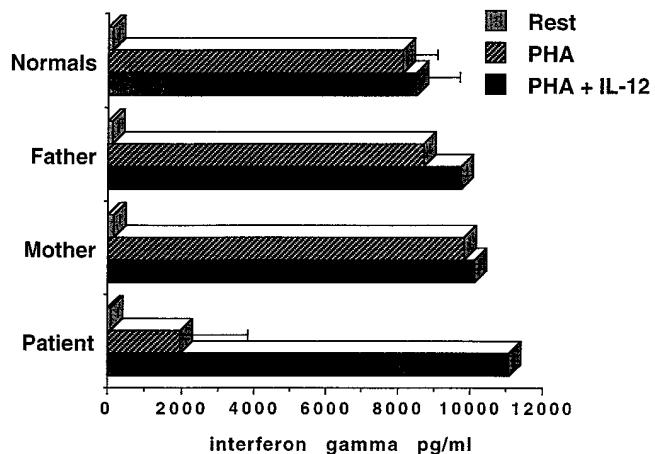


Figure 1. IFN- γ production by PBMCs. Peripheral blood mononuclear cell IFN- γ production in response to stimulation with PHA (hatched bars), or PHA plus IL-12 (PHA + IL-12; solid bars) in the patient, his parents, and normal controls. IFN- γ production from unstimulated cells (rest, gray bars). Normals, rest and PHA alone, n = 35; PHA + IL-12, n = 7. We examined the patient's blood on two separate occasions at rest and with PHA alone, and on one occasion with PHA + IL-12. The parents' blood was examined on one occasion. Data shown are mean \pm SE.

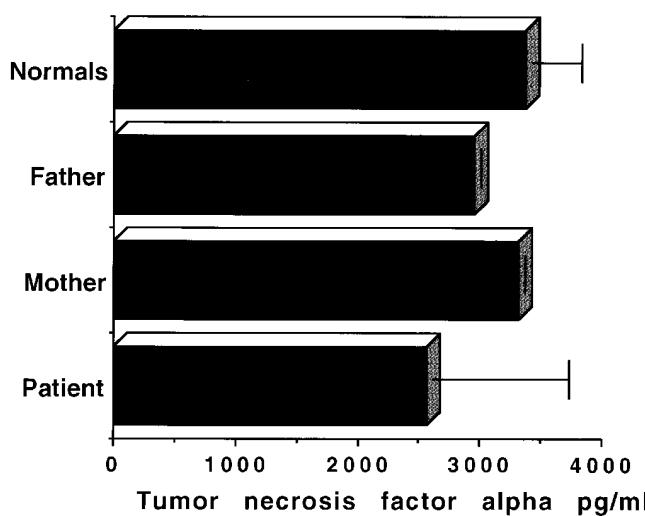


Figure 2. TNF- α production in response to stimulation with PHA in the patient, his parents, and normal controls ($n = 35$). The patient's blood was examined on two separate occasions. The parents' blood was examined on one occasion. Data shown are mean \pm SE.

duced normal amounts of TNF- α (Fig. 2). TNF- α production in response to endotoxin was also normal, indicating that the pathways involving CD14, the endotoxin receptor molecule on the surface of phagocytes, were normal (Fig. 3). However, when IFN- γ was added to endotoxin, the augmentation in TNF- α production seen in normal controls was not observed in the patient cells (normal controls, 5.37-fold increase, patient, 1.03-fold increase; Fig. 3). These data suggested that the PHA-induced TNF- α was being produced either by monocytes by an IFN- γ -independent mechanism, or by a cell population other than monocytes. Appropriate TNF- α production by patient cells under IFN- γ -independent conditions and failure to aug-

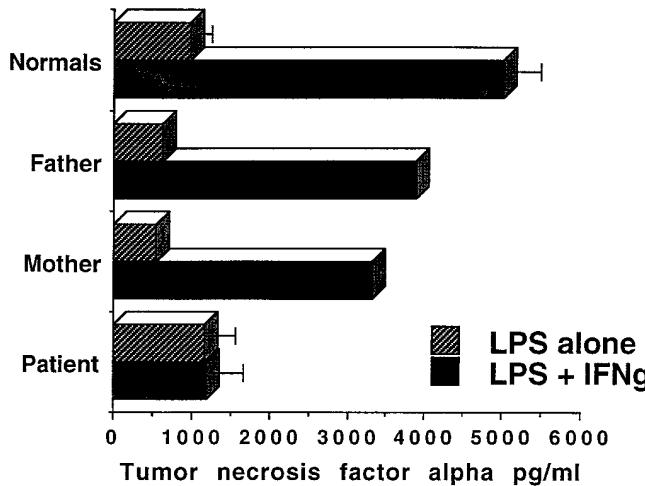


Figure 3. TNF- α production in response to LPS and IFN- γ . Stimulation with *Escherichia coli*-derived LPS (hatched bars), or LPS plus IFN- γ (LPS + IFN- γ ; solid bars) in the patient, his parents, and normal controls ($n = 35$). The patient's blood was examined on two separate occasions. The parents' blood was examined on one occasion. Data shown are mean \pm SE.

ment TNF- α production in response to IFN- γ suggested a defect in the IFN- γ receptor.

Monocyte surface expression of the ligand-binding chain of the IFN- γ receptor, IFN γ R1, was normal in distribution and intensity as detected by flow cytometry (Fig. 4). Efforts to detect IFN γ R2 on patient and normal cells by flow cytometry were unsuccessful. We detected messenger RNA for both IFN γ R1 and IFN γ R2 in patient PBMC by reverse transcription and PCR. Sequencing of the cDNA of IFN γ R1 showed no mutations. However, direct sequencing of a PCR product containing the open reading frame of the patient's signal transducing chain, IFN γ R2, showed a homozygous deletion of nucleotides 278 and 279 (Fig. 5 A). This dinucleotide deletion created a frame shift leading to a premature stop codon (TGA) at nucleotides 282-284 (Fig. 5 B). The deletion and stop codon are both located in exon III, which comprises a portion of the extracellular domain of IFN γ R2 (Fig. 5 C). To confirm this finding, we sequenced patient genomic DNA,

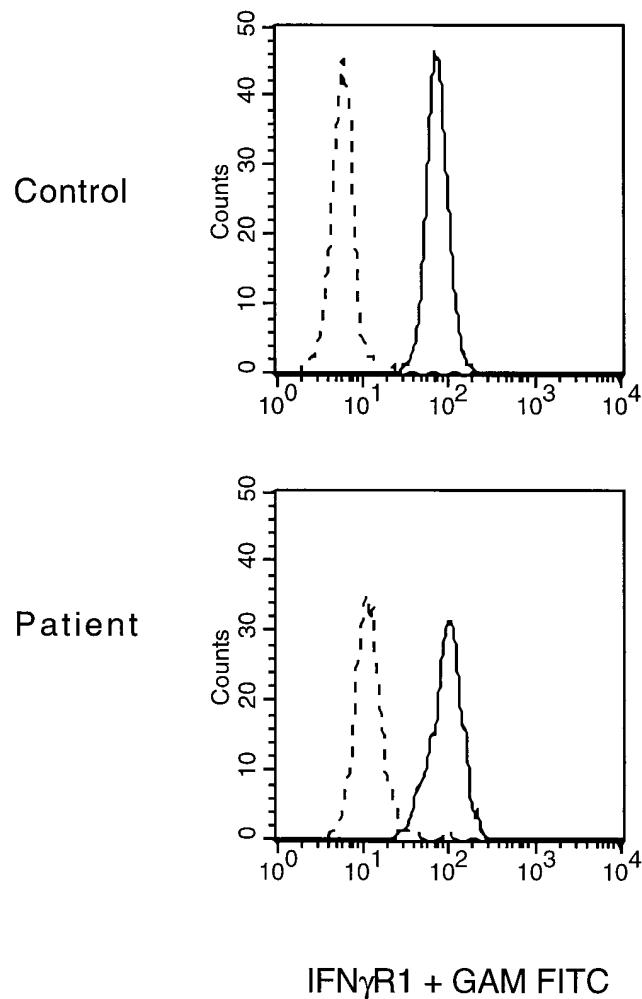


Figure 4. Detection of IFN- γ receptor 1 by flow cytometry. Expression of IFN- γ receptor 1 (IFN γ R1) on the surface of peripheral blood monocytes from the patient (bottom) and a representative normal control (top). Hatched line, Isotype control staining; solid line, IFN γ R1 specific antibody staining. GAM FITC, goat anti-mouse fluorescein isothiocyanate.

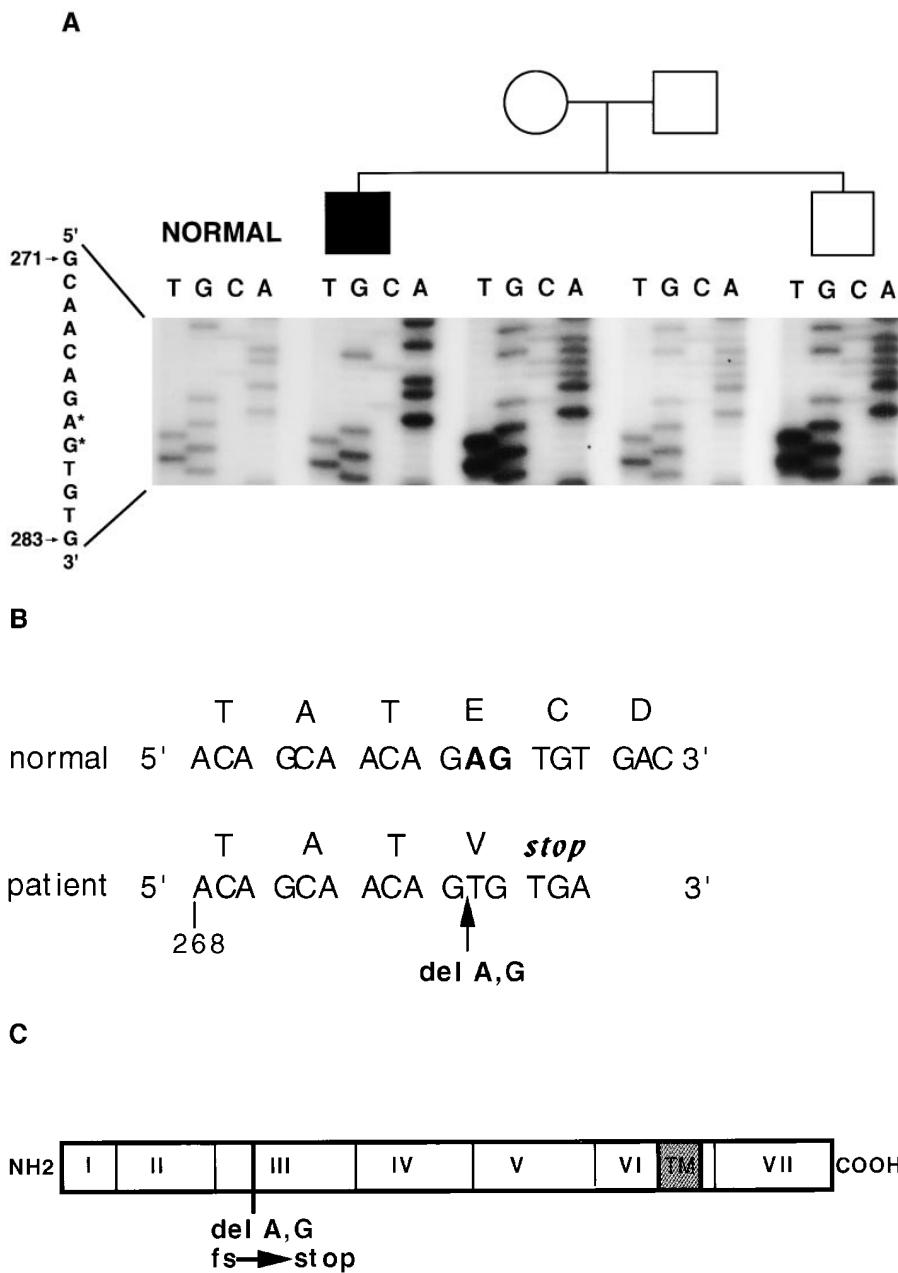


Figure 5. Mutation of the coding region of the IFN- γ receptor 2 (IFN γ R2) gene. **A** shows results of nucleotide sequence analysis of reverse transcriptase PCR products around nucleotides 278 and 279 of the complementary DNA for IFN γ R2 (numbered from the ATG translation initiation codon). From left to right: a normal control, the patient, his mother, father, and healthy sibling are shown under their respective symbols. (Squares, males; circles, females; shaded square, patient). **B** shows the complementary DNA sequence for the coding region around nucleotides 278 and 279. Single-letter amino acid abbreviations are shown above the nucleotide sequence. The deletion of nucleotides 278 and 279 results in a frame shift and a premature stop codon. **C** shows a schematic representation of IFN γ R2. Vertical lines, exons I-VII; shaded area, transmembrane (TM) domain. The dinucleotide deletion in the patient creates a frameshift (fs) and premature stop codon, predicting a truncated protein lacking a portion of exon III and all of exons IV through VII.

which showed the same mutation. We also sequenced PCR amplification products spanning exon III of cDNA from the patient's mother, father, and brother. These individuals each had two populations of PCR products, one corresponding to the normal sequence, and one with the same two nucleotide deletions as the patient, consistent with the heterozygous carrier state (Fig. 5A).

Activation of Stat1 by phosphorylation is required for IFN- γ signal transduction (8). We analyzed Stat1 activation in EBV-transformed B cells from the patient and a normal after IFN- γ stimulation. As Fig. 6 shows, phosphorylated Stat1 was detectable from IFN- γ -stimulated B cells from a normal control, but not from IFN- γ -stimulated patient B cells. Phosphorylated Stat1 was not detectable from unstimulated patient B cells or unstimulated normal control B cells. To confirm uniform load-

ing of cell lysates, the membrane was stripped and reprobed with a monoclonal antibody to Stat1. Together, these data show that Stat1 is present in patient B cells, but it is not phosphorylated in response to IFN- γ stimulation.

Discussion

In this child with disseminated atypical mycobacterial infection, in vitro PBMC-stimulation studies showed failure to augment TNF- α production in response to IFN- γ . Fluorescence-activated cell sorting analysis showed presence of IFN γ R1, but phosphorylation of Stat1 was absent after IFN- γ stimulation. These abnormalities pointed toward a defect in the signal-transducing chain of the IFN- γ receptor. We have demonstrated a protein chain-terminating mutation in the signaling

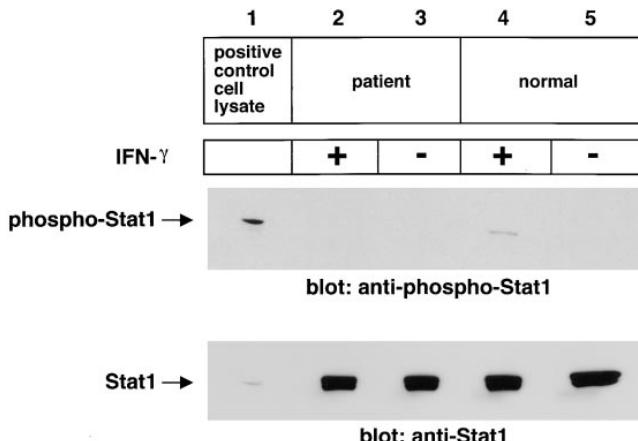


Figure 6. Western immunoblotting for phosphorylated Stat1. *Top*, rabbit antibody directed against phosphorylated Stat1. *Bottom*, the same blot stripped and reprobed with mouse anti-Stat1 to show equivalence of Stat1 loading in the respective lanes. Lane 1, positive control cell lysate containing phosphorylated Stat1; lane 2, patient cells stimulated with IFN- γ ; lane 3, unstimulated patient cells; lane 4, normal cells stimulated with IFN- γ ; lane 5, unstimulated normal cells.

chain of the IFN- γ receptor, IFN γ R2, as a novel cause for this immunodeficiency.

The IFN- γ receptor is composed of at least two subunits, IFN γ R1 and IFN γ R2, both of which are transmembrane proteins. The structure of the IFN- γ receptor complex and the role of its components in signal transduction have been well described (7–9). The binding of homodimeric IFN- γ to IFN γ R1 causes oligomerization of the two receptor subunits. Each receptor subunit is associated with a distinct Janus kinase (Jak), which is activated by reciprocal transphosphorylation upon oligomerization of IFN γ R1 and IFN γ R2. Jak activation then causes phosphorylation of IFN γ R1. A latent transcription factor, Stat1, binds to the phosphorylated IFN γ R1, undergoes tyrosine phosphorylation, and forms homodimers that translocate to the nucleus and regulate transcription of IFN- γ -inducible genes. Using cell lines expressing different components of the IFN- γ receptor complex, Kotenko et al. have shown that the association of IFN γ R1 with IFN γ R2 is necessary for signal transduction, and that the intracellular domain of IFN γ R2 is required for this function (7). Therefore, for our patient, if mRNA is stable and truncated IFN γ R2 is made, this protein would not be expected to support signal transduction. The absence of phosphorylated Stat1 in response to IFN- γ was consistent with lack of signal transduction by the IFN- γ receptor complex.

In our purified PBMC cytokine production assay, there was no appreciable IFN- γ -stimulated increase in TNF- α production in our patient, and we could detect no heterozygous phenotype in his parents. However, this does not exclude the possibility that there exists a heterozygous phenotype with respect to mycobacterial killing or other monocyte-macrophage functions. Levin et al. (10) and Newport et al. (1) found modest IFN- γ -induced increases in TNF- α production in the IFN γ R1-deficient patients, and intermediate TNF- α production in their heterozygous parents. These disparate findings may reflect differences in defects associated with IFN γ R1 ver-

sus IFN γ R2, or differences in the assay systems used. Reported clinical response to IFN- γ administration in three patients reported by Newport et al. (1) and lack of clinical response in our patient suggest that the in vitro observations may reflect real biological differences.

Our patient's clinical course is noteworthy for the fact that he has maintained his weight along the 25th percentile despite disseminated mycobacterial infection. His ability to maintain growth and development along normal lines during progressive infection is not typical of disseminated nontuberculous mycobacterial infections in HIV-uninfected hosts. We have treated several other children with disseminated *M. avium* complex infections and intact IFN- γ responses whose growth has been retarded during progressive infection (11, and S.M. Holland, unpublished observations). The lack of wasting observed in this patient may be due to the defect in IFN- γ signal transduction. TNF- α is a leading candidate for causing infection-related wasting in patients with chronic infections (12). Normal TNF- α production in vitro in this patient in response to LPS and PHA suggests that IFN- γ -induced upregulation of TNF- α may be important in the wasting response seen in chronic infections.

Elucidation of the pathways involved in host defense against mycobacteria is critical for understanding why some individuals exposed to these organisms develop disease, while other exposed individuals do not. It is unlikely that the majority of individuals with mycobacterial disease completely lack IFN- γ receptor proteins, given the severity of disease associated with the genotypes described to date. However, more subtle abnormalities in IFN- γ -mediated immune response may be more generally relevant, particularly in individuals with disease due to more virulent mycobacteria such as *M. tuberculosis*. Recently, Jouanguy et al. described partial IFN γ R1 deficiency in a patient with apparent pulmonary tuberculosis that responded well to conventional treatment, who also had a sibling with disseminated BCG (13).

Mice with disruptions of IFN- γ or IFN γ R1 genes are susceptible to experimental infection with mycobacteria (14–17) and other intracellular pathogens including parasites (18–21), viruses (22–24) and bacteria such as *Listeria monocytogenes* (25, 26) and *Legionella pneumophila* (27). In humans, salmonella infections have been described in two children with IFN γ R1 deficiency (1, 13) and probable legionella infection has been reported in one child (13), indicating that IFN- γ is probably important in the control of intracellular pathogens other than mycobacteria. This issue will be clarified as more patients with IFN- γ receptor defects are recognized.

Abnormalities in IFN- γ production (11), IL-12 production (28), IFN- γ binding (1–3, 13), and IFN- γ signaling (this report) have all identified the central importance of IFN- γ -generating and response pathways in the control of mycobacterial infections. These gene defects highlight the pathways that are important targets for immune-based therapies for mycobacterial disease.

Acknowledgments

We thank Dr. Glenn Billman and Dr. John Bastian for referral and care of the patient; Ms. Margaret R. Brown and Dr. Thomas A. Fleisher for flow cytometry; Dr. David Frucht for technical advice; and Dr. John Gallin and Dr. Jennifer Puck for critical review of the manuscript.

References

1. Newport, M.J., C.M. Huxley, S. Huston, C.M. Hawrylowicz, B.A. Oostra, R. Williamson, and M. Levin. 1996. A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection. *N. Engl. J. Med.* 335: 1941–1949.

2. Jouanguy, E., F. Altare, S. Lamhamedi, P. Revy, J.-F. Emile, M. Newport, M. Levin, S. Blanche, E. Seboun, A. Fischer, and J.-L. Casanova. 1996. Interferon-gamma-receptor deficiency in an infant with fatal *Bacille Calmette-Guérin* infection. *N. Engl. J. Med.* 335: 1956–1961.

3. Pierre-Audigier, C., E. Jouanguy, S. Lamhamedi, F. Altare, J. Rauzier, V. Vincent, D. Canioni, J.-F. Emile, A. Fischer, S. Blanche, J.-L. Gaillard, and J.-L. Casanova. 1997. Fatal disseminated *Mycobacterium smegmatis* infection in a child with inherited interferon gamma receptor deficiency. *Clin. Infect. Dis.* 24: 982–984.

4. Aguet, M., Z. Dembic, and G. Merlin. 1988. Molecular cloning and expression of the human interferon-gamma receptor. *Cell* 55: 273–280.

5. Soh, J., R.J. Donnelly, and S. Kotenko. 1994. Identification and sequence of an accessory factor required for activation of the human interferon gamma receptor. *Cell* 76: 793–802.

6. Rhee, S., C. Ebensperger, Z. Dembic, and S. Pestka. 1996. The structure of the gene for the second chain of the human interferon-gamma receptor. *J. Biol. Chem.* 271: 28947–28952.

7. Kotenko, S.V., L.S. Izotova, B.P. Pollack, T.M. Mariano, R.J. Donnelly, G. Muthukumaran, J.R. Cook, G. Garotta, O. Silvennoinen, J.N. Ihle, and S. Pestka. 1995. Interaction between the components of the interferon gamma receptor complex. *J. Biol. Chem.* 270: 20915–20921.

8. Darnell, J.E., Jr., I.M. Kerr, and G.R. Stark. 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 264: 1415–1421.

9. Bach, E.A., M. Aguet, and R.D. Schreiber. 1997. The IFN gamma receptor: a paradigm for cytokine receptor signaling. *Annu. Rev. Immunol.* 15: 563–591.

10. Levin, M., M.J. Newport, S. D’Souza, P. Kalabalikis, I.N. Brown, H.M. Lenicker, P.V. Agius, E.G. Davies, A. Thrasher, N. Klein, and J.M. Blackwell. 1995. Familial disseminated atypical mycobacterial infection in childhood: a human mycobacterial susceptibility gene? *Lancet* 345: 79–83.

11. Holland, S.M., E.M. Eisenstein, D.B. Kuhns, M.L. Turner, T.A. Fleisher, W. Strober, and J.I. Gallin. 1994. Treatment of refractory disseminated nontuberculous mycobacterial infection with interferon gamma: a preliminary report. *N. Engl. J. Med.* 330: 1348–1355.

12. Beutler, B., and A. Cerami. 1986. Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature* 320: 584–588.

13. Jouanguy, E., S. Lamhamedi-Cherradi, F. Altare, M.-C. Fondaneche, D. Tuerlinckx, S. Blanche, J.-F. Emile, J.-L. Gaillard, R. Schreiber, M. Levin, A. Fischer, C. Hivroz, and J.-L. Casanova. 1997. Partial interferon- γ receptor 1 deficiency in a child with tuberculoid bacillus Calmette-Guérin infection and a sibling with clinical tuberculosis. *J. Clin. Invest.* 100: 2658–2664.

14. Cooper, A.M., D.K. Dalton, T.A. Stewart, J.P. Griffin, D.G. Russell, and I.M. Orme. 1993. Disseminated tuberculosis in interferon gamma gene-disrupted mice. *J. Exp. Med.* 178: 2243–2247.

15. Dalton, D.K., S. Pitts-Meek, S. Keshav, I.S. Figari, A. Bradley, and T.A. Stewart. 1993. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. *Science* 259: 1739–1742.

16. Kamijo, R., J. Le, D. Shapiro, E.A. Havell, S. Huang, M. Aguet, M. Bolland, and J. Vilcek. 1993. Mice that lack the interferon-gamma receptor have profoundly altered responses to infection with *Bacillus Calmette-Guérin* and subsequent challenge with lipopolysaccharide. *J. Exp. Med.* 178: 1435–1440.

17. Flynn, J.L., J. Chan, K.J. Triebold, D.K. Dalton, T.A. Stewart, and B.R. Bloom. 1993. An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. *J. Exp. Med.* 178: 2249–2254.

18. Garry, B.A., R.A. Ezekowitz, and A.G. Harmsen. 1997. Role of gamma interferon in the host immune and inflammatory responses to *Pneumocystis carinii* infection. *Infect. Immun.* 65: 373–379.

19. Scharton-Kersten, T.M., T.A. Wynn, E.Y. Denkers, S. Bala, E. Grunwald, S. Hieny, R.T. Gazzinelli, and A. Sher. 1996. In the absence of endogenous IFN-gamma, mice develop unimpaired IL-12 responses to *Toxoplasma gondii* while failing to control acute infection. *J. Immunol.* 157: 4045–4054.

20. Wang, Z.E., S.L. Reiner, S. Zheng, D.K. Dalton, and R.M. Locksley. 1994. CD4+ effector cells default to the TH2 pathway in IFN-gamma-deficient mice infected with *Leishmania major*. *J. Exp. Med.* 179: 1367–1371.

21. Swihart, K., U. Fruth, N. Messmer, K. Hug, R. Behin, S. Huang, G. Del Giudice, M. Aguet, and J.A. Louis. 1995. Mice from a genetically resistant background lacking the interferon gamma receptor are susceptible to infection with *Leishmania major* but mount a polarized T helper cell 1-type CD4+ T cell response. *J. Exp. Med.* 181: 961–971.

22. Fiette, L., C. Aubert, U. Muller, S. Huang, M. Aguet, M. Brahic, and J.F. Bureau. 1995. Theiler’s virus infection of 129Sv mice that lack the interferon alpha/beta or interferon gamma receptors. *J. Exp. Med.* 181: 2069–2076.

23. Bouley, D.M., S. Kanangat, W. Wire, and B.T. Rouse. 1995. Characterization of herpes simplex virus type-1 infection and herpetic stromal keratitis development in IFN-gamma knockout mice. *J. Immunol.* 155: 3964–3971.

24. Yu, Z., E. Manickan, and B.T. Rouse. 1996. Role of interferon-gamma in immunity to herpes simplex virus. *J. Leukocyte Biol.* 60: 528–532.

25. Harty, J.T., and M.J. Bevan. 1995. Specific immunity to *Listeria monocytogenes* in the absence of IFN-gamma. *Immunity* 3: 109–117.

26. Szalay, G., C.H. Ladel, C. Blum, and S.H. Kaufmann. 1996. IL-4 neutralization or TNF-alpha treatment ameliorate disease by an intracellular pathogen in IFN-gamma receptor-deficient mice. *J. Immunol.* 157: 4746–4750.

27. Heath, L., C. Chrisp, G. Huffnagle, M. LeGendre, Y. Osawa, M. Hurley, C. Engleberg, J. Fantone, and J. Brieland. 1996. Effector mechanisms responsible for gamma interferon-mediated host resistance to *Legionella pneumophila* lung infection: the role of endogenous nitric oxide differs in susceptible and resistant murine hosts. *Infect. Immun.* 64: 5151–5160.

28. Frucht, D.M., and S.M. Holland. 1996. Defective monocyte costimulation for IFN-gamma production in familial disseminated *Mycobacterium avium* complex infection: abnormal IL-12 regulation. *J. Immunol.* 157: 411–416.