Partial Interferon-γ Receptor Signaling Chain Deficiency in a Patient with Bacille Calmette-Guérin and Mycobacterium abscessus Infection

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Complete deficiency of either of the two human interferon (IFN)–γ receptor components, the ligand-binding IFN-γR1 chain and the signaling IFN-γR2 chain, is invariably associated with early-onset infection caused by bacille Calmette-Guérin vaccines and/or environmental nontuberculous mycobacteria, poor granuloma formation, and a fatal outcome in childhood. Partial IFN-γR1 deficiency is associated with a milder histopathologic and clinical phenotype. Cells from a 20-year-old healthy person with a history of curable infections due to bacille Calmette-Guérin and Mycobacterium abscessus and mature granulomas in childhood were investigated. There was a homozygous nucleotide substitution in IFNGR2, causing an amino acid substitution in the extracellular region of the encoded receptor. Cell surface IFN-γR2 were detected by flow cytometry. Cellular responses to IFN-γ were impaired but not abolished. Transfection with the wild-type IFNGR2 gene restored full responsiveness to IFN-γ. This is the first demonstration of partial IFN-γR2 deficiency in humans.

In patients with Mendelian susceptibility to mycobacterial infection (MIM 209950) [1], weakly virulent species, such as bacille Calmette-Guérin (BCG) attenuated vaccines and environmental nontuberculous mycobacteria, may cause severe disease. Pathogenic mutations have been identified in IFNGR1 [2–9] and IFNGR2 [10], encoding the two subunits of the receptor for interferon (IFN)–γR1 and –γR2, a pleiotropic cytokine secreted mostly by NK and T cells.

Three types of IFNGR1 mutation, each associated with a different form of IFN-γR1 deficiency, have been described. Complete IFN-γR1 deficiency is caused by a variety of recessive null mutations in the 5′ region of the IFNGR1 gene [2–4, 6–8]. Partial IFN-γR1 deficiency due to a recessive missense mutation in the same region, which encodes the extracellular domain, has been reported elsewhere [5]. A different form of partial IFN-γR1 deficiency is caused by dominant mutations in the 3′ region of the gene, which encodes the intracytoplasmic region [9].

There is a correlation between the IFNGR1 genotype and the cellular, histopathologic, and clinical phenotype [9]. Complete IFN-γR1 deficiency is associated with early-onset nontuberculous mycobacterial infection, a lack of response to IFN-γ therapy, and a fatal outcome in childhood. Tissue granulomas are poorly delimited and differentiated and are often multicavitary. Partial IFN-γR1 deficiency is associated with late-onset nontuberculous mycobacterial infection and a much better prognosis. The tissue granulomas formed in response to BCG are well delimited and differentiated and often pauciba-
ciliary, even though granulomatous lesions in response to non-tuberculous mycobacterial infections are generally immature.

One patient with complete IFN-γR2 deficiency has been described [10]. This patient had severe disseminated Mycobacterium avium infection in early childhood with no mature granulomas. Complete IFN-γR1 and IFN-γR2 defects therefore seem to be responsible for the same severe cellular, histopathologic, and clinical phenotype. We have now studied a 20-year-old patient with a milder clinical and histopathologic phenotype. We tested the patient’s cells for early (nuclear translocation of STAT-1) and late (surface expression of HLA-DR) responses to IFN-γ and sequenced the genes encoding both IFN-γ receptor chains and found that this patient has partial IFN-γR2 deficiency.

Patient and Methods

Case report. The child was born in France to first-cousin Portuguese parents. She was vaccinated shortly after birth with live BCG and developed disseminated BCG infection (patient 8 in [11]). Mature granulomas were seen (patient 7 in [12]). The patient was successfully treated with antimycobacterial drugs, which were discontinued at 4 years of age. At age 16 years, she developed cellulitis and adenitis of the neck, with disseminated skin vasculitis, wasting, fever, leukocytosis, and high levels of inflammation markers. Skin and lymph-node biopsies showed mature granulomas. No acid-fast bacilli were observed, but M. abscessus was cultured from the lesions. Antimycobacterial drugs, although efficient in vitro against the M. abscessus isolate, were not sufficient to control the mycobacterial disease process, and only when subcutaneous treatment with IFN-γ was added did definitive clinical improvement occur. After 7 months, IFN-γ was discontinued, and, after 3 years, antibiotics were also discontinued. The patient is now 20 years old and doing well, receiving no prophylactic therapy. No other unusual infections were noted, and specific antibodies against other intracellular agents, such as Chlamydia pneumoniae, varicella-zoster virus, and Epstein-Barr virus (EBV), were detected in the serum. No antibodies specific for human immunodeficiency virus type 1 were found. Her healthy younger brother has been vaccinated with live BCG with no adverse effects.

Molecular genetics. Microsatellites, IFNGRI and IFNGR2 exons, and cDNAs from EBV-transformed B cells and peripheral blood mononuclear cells (PBMC) from the patient herein investigated, her parents and sibling, and 60 unrelated Caucasian healthy persons, were amplified as described elsewhere [6, 9, 13]. IFNGR2 exon 3 was amplified with use of the following primers: 5'-ATTCTGTGAATTGAAATCCT-3' (sense) and 5'-TGAAGAAAACCTGGAAATACT-3' (antisense). The polymerase chain reaction products were directly sequenced with Thermosequenase (Amersham, Amersham, UK) [3, 9].

Electrophoretic mobility shift assay. EBV-transformed B cells from the patient herein investigated, 10 healthy persons, a patient with complete IFN-γR1 deficiency [5], a patient with partial recessive IFN-γR1 deficiency [5], a patient with partial dominant IFN-γR1 deficiency [9], and a patient with complete IFN-γR2 deficiency [10] were activated by incubation for 30 min with various concentrations of IFN-γ (Biogenex Laboratories, San Ramon, CA) or IFN-α (Schering-Plough, Liberty Corner, NJ) [5]. The mobility shift assay was done with 1 μg of nuclear extract and a 32P-end-labeled double-stranded DNA probe corresponding to the IFN-γ response region. The supershift assay was done with the STAT-1-specific antibody C24 (Santa Cruz Biotechnology, Santa Cruz, CA) [9].

Flow cytometry and IFN-γ binding assay. PBMC and EBV-transformed B cells from the patient herein investigated, a healthy person, and a patient with complete IFN-γR2 deficiency [10] were stained with a specific anti-human IFN-γR2 mouse IgG2a monoclonal antibody (C.11), followed by fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG (Dako, Carpinteria, CA) [14]. Alternatively, an IgG2b mouse monoclonal antibody specific for human IFN-γR1 (GIR-94) was used elsewhere [9]. Cells were analyzed on a FACScan flow cytometer by use of Lysis-II software (Becton Dickinson, Baltimore). Specific binding of 125I-labeled IFN-γ to cell surface IFN-γR1 molecules on EBV-transformed B cells was quantified as described [9].

HLA-DR induction on fibroblasts and transfection. One million SV40T-transformed fibroblasts from the patient herein investigated, a healthy person, and a patient with complete IFN-γR1 deficiency [6] were incubated with 50, 500, or 5000 IU/mL recombinant IFN-γ (Biogenex Laboratories) for 48 h. HLA-DR expression was then analyzed by flow cytometry or fluorescence microscopy by use of a monoclonal fluorescein isothiocyanate-labeled anti-human HLA-DR antibody (Becton Dickinson) [5, 9]. Fibroblasts were transiently transfected [6] by use of the EMCV.SR expression vector encoding IFNGRI or IFNGR2 [9]. After transfection, HLA-DR expression was determined by fluorescence microscopy with specific antibodies [6].

Results

IFN-γ signaling is mediated by the translocation of activated STAT-1 homodimers into the nucleus, where they bind specific DNA-recognition sequences to initiate gene transcription. We investigated the responsiveness of the patient’s cells to IFN-γ by analyzing the nuclear translocation of STAT-1 in EBV-transformed B cells by use of an electrophoretic mobility shift assay (figure 1A). The amount of STAT-1 in nuclear extracts from a healthy person increased with the concentration of IFN-γ, but a plateau was reached by 1000 IU/mL. No significant differences were detected between 10 healthy persons (not shown). Levels of STAT-1 translocation detected in the patient’s cells in response to all concentrations of IFN-γ were consistently lower (10% of control). The molecules detected were confirmed to be STAT-1 by supershift assays (not shown). A normal dose response to IFN-α was observed with use of the same DNA probe, suggesting that the patient’s cells were not globally unresponsive (not shown). This pattern was unique among a series of B cell lines from patients with known complete recessive IFN-γR1 or IFN-γR2 deficiency [3, 10] or partial recessive or dominant IFN-γR1 deficiency [5, 9].

Mutations causing IFN-γR1 deficiency were excluded on several grounds. Intrafamilial segregation of polymorphic micro-
Figure 1.  

A, Impaired nuclear translocation of STAT-1 in patient’s B cells stimulated by interferon (IFN)-γ. In electrophoretic mobility shift assay (1 of 4 separate experiments), Epstein-Barr virus (EBV)-transformed B cells from patient investigated (P), patient with complete IFN-γR2 deficiency (C−), and healthy control subject (C+) were tested for nuclear translocation of STAT-1 in response to various doses of IFN-γ. Competition with cold probe is indicated (E).  

B, Normal binding of IFN-γ to cell surface. In binding assay, EBV-transformed B cells from patient and control were incubated with various concentrations of 125I-labeled IFN-γ to determine number (, and , respectively) and affinity (, and mean ± SD 8.57 × 10⁷ ± 1.88 × 10⁷ and 1.13 × 10⁸ ± 1.03 × 10⁸, respectively) of IFN-γ binding sites on cell surface by Scatchard analysis.  

C, Identification of IFNGR2 R114C mutation and intrafamilial segregation of R114C IFNGR2 allele. Nucleotide substitution at position 340 in exon 3 (C>T) results in amino acid substitution at position 114 (R→C; missense mutation designated as R114C; arrow). Parents are heterozygous for R114C IFNGR2 allele, patient is homozygous for R114C allele, and her sibling is homozygous for wild-type allele.  

D, Structure of IFNGR2 open-reading frame and position of R114C mutation. Exons (roman numerals), leader (L), extracellular (EC), transmembrane (TM), and intracellular (IC) regions are indicated.
Figure 2.  
A. Defective induction of surface HLA-DR molecules on patient’s fibroblasts in response to interferon (IFN)-γ. Fibroblasts from patient (P) and control subject (C) were incubated with various concentrations of IFN-γ for 48 h, stained with HLA-DR–specific antibody, and analyzed by flow cytometry.  
B. Complementation with wild-type IFNGR2. Fibroblasts from patient were transiently transfected with wild-type IFNGR1 (left) or IFNGR2 (right) and incubated with 500 IU/mL IFN-γ. HLA-DR was detected on cell surface with a specific antibody by immunofluorescence [6].  
C. Normal expression of IFN-γR2 on Epstein-barr virus (EBV)–transformed B cells. EBV-transformed B cells from patient (P), healthy control (C+), and patient with complete recessive IFN-γR2 deficiency (C−) were stained with IFN-γR2–specific C.11 monoclonal antibody.
satellites within (FA1) or flanking (D6S1009 and D6S1587) the IFNGRI gene [6] revealed that the patient was heterozygous for all three markers (not shown). This is not consistent with the inheritance of a common pathogenic IFNGRI allele from two consanguineous parents. No mutations were found by sequencing of the seven IFNGRI exons and flanking intron regions (not shown). Surface expression of IFN-γR1 on PBMC and EBV-transformed B cells was normal, as detected by flow cytometry with the GIR-94 antibody (not shown). The affinity and number of IFN-γ–specific binding sites on the surface of EBV-transformed B cells were also normal (figure 1B). These data exclude the possibility of cis IFNGRI mutations and trans mutations of other regulatory genes potentially controlling IFN-γR1 expression in this patient.

Amplification of the IFNGR2 open-reading frame from blood and EBV-transformed B cell cDNAs gave a single product of the expected molecular weight (1 kb), as detected by agarose gel electrophoresis (not shown). Direct sequencing revealed a C→T nucleotide substitution at position 340, resulting in an Arg→Cys amino acid substitution at position 114 in the extracellular region. Homozygosity for the mutation was confirmed by sequencing of an exon 3 polymerase chain reaction product amplified from genomic DNA from PBMC of the patient [13] (figure 1C). No other mutations were found in the exons and flanking intron regions, and the IFNGR2 allele was therefore designated as R114C (figure 1D). This mutation was not found in the patient’s sibling and 60 healthy Caucasians investigated. The parents were found to be heterozygous for R114C and a wild-type IFNGR2 allele (figure 1C). The IFNGR2 R114C allele is therefore inherited as a recessive trait that segregates with the clinical syndrome.

HLA class II molecules are inducible by IFN-γ in nonprofessional antigen-presenting cells. We tested the induction of HLA-DR at the cell surface of SV40-transformed fibroblasts from the patient. No expression was detected in response to 50 and 500 IU/mL IFN-γ, in contrast to fibroblasts from a healthy person (figure 2A). However, HLA-DR was detected in response to 5000 IU/mL (at lower levels than in control fibroblasts; figure 2A), whereas it was not detected on fibroblasts from a patient with complete IFN-γR1 deficiency (not shown) [6]. Thus, the IFN-γ responsiveness of both B cells (early STAT-1 nuclear translocation) and fibroblasts (late HLA-DR surface expression) was impaired but not abolished. Transient transfection of the patient’s fibroblasts with wild-type IFNGR2 restored responsiveness of ~5% of cells to 500 IU/mL IFN-γ (figure 2B). In contrast, transfection with an insertless vector or wild-type IFNGRI did not restore responsiveness of any cell (figure 2B). Thus, the impaired cell response to IFN-γ was due to defective IFN-γR2 molecules.

We detected membrane-bound IFN-γR2 in EBV-transformed B cells from the patient and a control subject by flow cytometry with the specific C.11 monoclonal antibody (figure 2C). No IFN-γR2 were detected on the surface of intact B cells from a patient with complete IFN-γR2 deficiency (figure 2C) [10]. IFN-γR2 molecules were also detected on the surface of fresh monocytes from the patient and a control subject (not shown). Thus, the mutation does not prevent expression of the molecule at the cell surface. These data strongly suggest that there is no other undetected disease-causing regulatory mutation in the IFNGR2 gene and that there is a causal relationship between homozygosity for the R114C IFNGR2 allele and impaired, but not abolished, cell responses to IFN-γ in the patient.

Discussion

We report herein the first patient with partial, as opposed to complete [10], IFN-γR2 deficiency. There is a homozygous single amino acid substitution (R114C) in the extracellular region of IFN-γR2, which does not prevent cell-surface expression of the receptor. The mutant receptors are poorly functional, because early events following stimulation with IFN-γ, such as STAT-1 nuclear translocation, and late events, such as HLA-DR surface expression, are impaired. The functional defect is partial, unlike a previously reported patient with complete IFN-γR2 deficiency [10]. No cell surface IFN-γR2 molecules were detected in the latter patient (this report).

What is the molecular mechanism underlying the partial loss of responsiveness of our patient’s cells to IFN-γ? The number and affinity of cell surface IFN-γ–binding sites are normal, suggesting that the mutation of IFN-γR2 does not interfere with IFN-γ binding and confirming IFN-γR1 as the cytokine-binding chain [15]. Mutant IFN-γR2 molecules may have a lower affinity for IFN-γR1, thereby decreasing their recruitment after dimerization of IFN-γR1 by IFN-γ, leading to a decrease in the number of functional complexes.

Our results suggest that there is a correlation between the IFNGR2 genotype and the cellular, histopathologic, and clinical phenotype. The R114C IFNGR2 mutation impairs, but does not abolish, the cell response to IFN-γ and is associated with curable BCG infection and late-onset M. abscessus infection, with mature paucibacillary granulomas in response to both mycobacterial species. The patient is now 20 years old and doing well with no prophylactic treatment. The null homozygous IFNGR2 mutation previously reported in another patient [10] abolishes cell responsiveness to IFN-γ and is associated with early-onset and severe M. avium infection with no mature granulomas. The genotype-phenotype correlation previously established for IFNGRI [5, 9] thus also applies to IFNGR2. The level of IFN-γ–mediated immunity seems to be the crucial factor determining the histopathologic lesions associated with, and the clinical outcome of, mycobacterial infections.

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References


