

Partial Interferon- γ Receptor 1 Deficiency in a Child with Tuberculoïd Bacillus Calmette-Guérin Infection and a Sibling with Clinical Tuberculosis

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Abstract

Complete interferon- γ receptor 1 (IFN γ R1) deficiency has been identified previously as a cause of fatal bacillus Calmette-Guérin (BCG) infection with lepromatoid granulomas, and of disseminated nontuberculous mycobacterial (NTM) infection in children who had not been inoculated with BCG. We report here a kindred with partial IFN γ R1 deficiency: one child afflicted by disseminated BCG infection with tuberculoïd granulomas, and a sibling, who had not been inoculated previously with BCG, with clinical tuberculosis. Both responded to antimicrobials and are currently well without prophylactic therapy. Impaired response to IFN- γ was documented in B cells by signal transducer and activator of transcription 1 nuclear translocation, in fibroblasts by cell surface HLA class II induction, and in monocytes by cell surface CD64 induction and TNF- α secretion. Whereas cells from healthy children responded to even low IFN- γ concentrations (10 IU/ml), and cells from a child with complete IFN γ R1 deficiency did not respond to even high IFN- γ concentrations (10,000 IU/ml), cells from the two siblings did not respond to low or intermediate concentrations, yet responded to high IFN- γ concentrations. A homozygous missense *IFNGR1* mutation was identified, and its pathogenic role was ascertained by molecular complementation. Thus, whereas complete IFN γ R1 deficiency in previously identified kindreds caused fatal lepromatoid BCG infection and disseminated NTM infection, partial IFN γ R1 deficiency in this kindred caused curable tuberculoïd BCG infection and clinical tuberculosis. (*J. Clin. Invest.* 1997. 100:2658–2664.) **Key words:** inherited disorder • immunodeficiency • granuloma • macrophage • mycobacteria

Introduction

Inherited IFN- γ receptor 1 chain (IFN γ R1)¹ deficiency has been identified recently in three kindreds (1–8).² The IFN γ R1-encoding gene (*IFNGR1*) mutations identified were found to preclude cell surface expression of the high affinity ligand-binding chain of the receptor, and to be responsible for a lack of cellular responses to IFN- γ . Complete IFN γ R1 deficiency determines a phenotype characterized by a severe, profound, and selective susceptibility to poorly pathogenic mycobacteria. In one kindred, a child with complete IFN γ R1 deficiency died of disseminated infection due to attenuated *Mycobacterium bovis*, bacillus Calmette-Guérin (BCG) strain, after inoculation of live BCG vaccine (1–4). In two other kindreds, the affected children, who had not been inoculated with BCG, developed severe and often fatal infections due to environmental nontuberculous mycobacteria (NTM), such as *Mycobacterium avium*, *Mycobacterium fortuitum*, *Mycobacterium chelonae*, and *Mycobacterium smegmatis* (5–8).²

Another feature of children with complete IFN γ R1 deficiency is their inability to develop mature granulomas in response to mycobacteria. In one child, BCG granulomas were found to be poorly circumscribed and poorly differentiated, consisting of widespread macrophages with no differentiation into epithelioid or giant cells and without surrounding lymphocytes (3, 4). The granulomatous lesions were multibacillary, with many visible acid-fast bacilli within macrophages, resembling mycobacterial granulomas observed in individuals with lepromatous leprosy. Likewise, NTM granulomas in other children with complete IFN γ R1 deficiency were poorly circumscribed and poorly differentiated, although there were fewer visible organisms (5, 7, 8).

To better characterize this inherited disorder, two important questions need to be addressed. First, the selective susceptibility to poorly pathogenic mycobacteria raises the question of whether IFN γ R1 deficiency may also predispose to mycobacterial infections, such as tuberculosis, due to more virulent spe-

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1. *Abbreviations used in this paper:* gko, gamma knockout; IFN γ R1, IFN- γ receptor 1 chain; *IFNGR1*, IFN γ R1-encoding gene; NTM, nontuberculous mycobacteria; STAT, signal transducer and activator of transcription.

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cies. Second, the association of complete IFN γ R1 deficiency with fatal lepromatoid BCG infection raises the question of whether curable disseminated BCG infections with tuberculoid, i.e., paucibacillary, well-differentiated, and well-circumscribed, mycobacterial granulomas (2, 3) may be associated with partial IFN γ R1 deficiency. Therefore, we have investigated a kindred with curable tuberculoid disseminated BCG infection in one child and clinical tuberculosis in a sibling who had not been inoculated previously with BCG.

Methods

Patients. The only two children born to consanguineous Portuguese parents were investigated. A boy vaccinated with BCG at 1 mo of age rapidly developed disseminated BCG infection (patient 10 in reference 2). No well-defined immune deficiency could be identified (2, 9). Skin and lymph node granulomas were tuberculoid, i.e., well-circumscribed and well-differentiated with epithelioid cells and no visible acid-fast bacilli, defining a type I idiopathic disseminated BCG infection (3) (Fig. 1; patient A10 in reference 3). He responded well to appropriate antimycobacterial therapy and was well until 6 yr of age, when he was diagnosed with disseminated *Salmonella enteritidis* which responded to a prolonged course of amoxicillin. He had two episodes of pneumonitis thought to be due to a legionella species and *Mycoplasma pneumoniae* on the basis of positive specific serology. He is currently clinically well without any prophylactic antibiotic regimen at 17 yr of age.

His younger sister was not vaccinated with BCG and was healthy until 3 yr of age, when she developed a persistent cough with fatigue and anorexia. She was found to have erythema nodosa and a lung infiltrate on her chest x-ray. Despite the absence of visible organisms or positive cultures, symptomatic primary tuberculosis was diagnosed on the basis of (a) the poor clinical response to common antibiotics, (b) the development of a strong positive delayed-type hypersensitivity to 10 IU of tuberculous protein-purified derivative (13-mm induration), (c) a rapid clinical improvement with antimycobacterial drugs directed at tuberculous species (rifampicin, isoniazid, and ethambutol), and (d) the lack of recurrence. A few weeks after her brother, she too developed a pneumonitis typical of *M. pneumoniae*, confirmed by appropriate specific antibody responses. She is also well at 13 yr of age without any prophylactic therapy. Both siblings have some atopy but no signs of autoimmunity.

Microsatellite analysis and IFN γ R1 gene sequencing. Microsatellites D6S1009, FA1, and D6S1587 are highly polymorphic markers

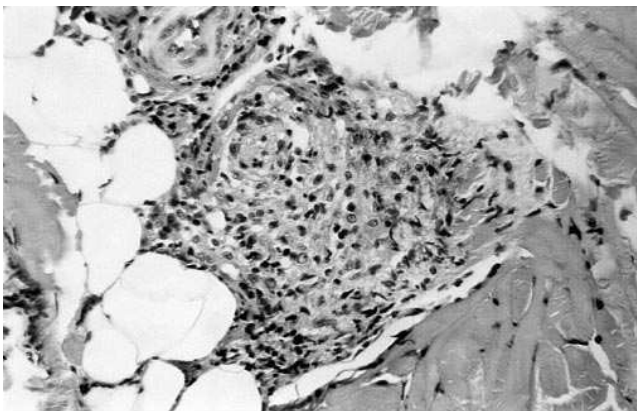


Figure 1. A tuberculoid BCG granuloma. A skin biopsy from a sibling described herein (the boy) shows a well-circumscribed granuloma which contains epithelioid cells (hematoxylin and eosin-stained; $\times 20$).

encompassing the *IFN γ R1* gene.² Conditions for PCR and sequencing of *IFN γ R1* cDNA have also been reported previously (4). Genomic PCR with primers specific for exon 3 (sense, 5'-CTG TGA ATA AAA AGC AAA GC-3', and antisense, 5'-AAA GCA AAC ATA CAG AAG AC-3') was achieved and sequenced.²

Expression of HLA class II on fibroblasts in response to IFN- γ . Detection of HLA class II molecules on fibroblasts by flow cytometry or fluorescent microscopy with specific antibodies was performed.² Transient transfection with wild-type or I87T *IFN γ R1* gene was also performed.²

Nuclear translocation of signal transducer and activator of transcription (STAT) 1 in B cells in response to IFN- γ . The protocol reported in reference 10 was applied to EBV-transformed B cells. Cells were activated with variable concentrations of IFN- γ and lysed on ice. After centrifugation, nuclei were resuspended in nuclear extraction buffer, vortexed, incubated on ice, and centrifuged. The protein concentration in the supernatant was determined, and mobility-shift assay was performed with 1 μ g of nuclear extract and a ³²P-end-labeled double-stranded DNA probe corresponding to the IFN- γ response region (10).

Expression of CD64 on monocytes in response to IFN- γ . Monocytes were harvested from PBMCs after adherence on petri dishes. Monocytes were scraped gently with a rubber policeman, plated into 75-mm easy-grip tissue culture dishes (Falcon Labware, Becton Dickinson, Oxnard, CA), and cultured for 24 h in the presence of variable concentrations of IFN- γ . Detection of cell surface CD64 on cultured monocytes was performed by flow cytometry with FITC-labeled specific antibody (Immunotech, Marseille, France) and isotypic control antibody.

Production of TNF- α by monocytes in response to LPS and IFN- γ . Quantification of TNF- α production by PBMCs in response to LPS and IFN- γ was adapted from a previously reported procedure (5). Briefly, PBMCs were incubated in culture medium at 37°C with variable concentrations of IFN- γ for 2 h before *Escherichia coli* endotoxin was added (final concentration, 10 μ g/ml). Incubation was continued for an additional 3 h, and TNF- α was assayed in the supernatant with an enzyme-linked immunoassay.

Flow cytometry with anti-IFN γ R1 antibodies. PBMCs were stained with five anti-IFN γ R1 antibodies, γ R38 and γ R99 (11), GIR-94 and GIR-208 (12), and 21-31.1 (Valbiotech, Paris, France), and with their respective isotypic control antibodies as described previously (4).

Results

Molecular diagnosis of IFN γ R1 deficiency. To investigate the role of the *IFN γ R1* gene in this family, we followed a procedure based on microsatellite segregation to screen candidate kindreds, a functional assay to question the diagnosis, gene sequencing to identify mutations, and molecular complementation to ascertain the pathogenic role of mutant alleles. We first studied the intrafamilial segregation of polymorphic microsatellites encompassing the *IFN γ R1* gene.² For the intragenic FA1 marker, the two siblings were found to be homozygous for the same allele (data not shown). This segregation was compatible with the diagnosis of IFN γ R1 deficiency by inheritance of a common pathogenic *IFN γ R1* allele from the two consanguineous parents.

To investigate this hypothesis further, we studied by flow cytometry the induction of HLA class II expression on SV40T-transformed fibroblasts in response to IFN- γ (Fig. 2 a). Addition of exogenous IFN- γ (100 IU/ml) was found to reproducibly induce HLA-II in fibroblasts from healthy individuals. No response to IFN- γ could be detected in fibroblasts from a child with complete IFN γ R1 deficiency.² Likewise, for the two siblings investigated, no response was observed, documenting an

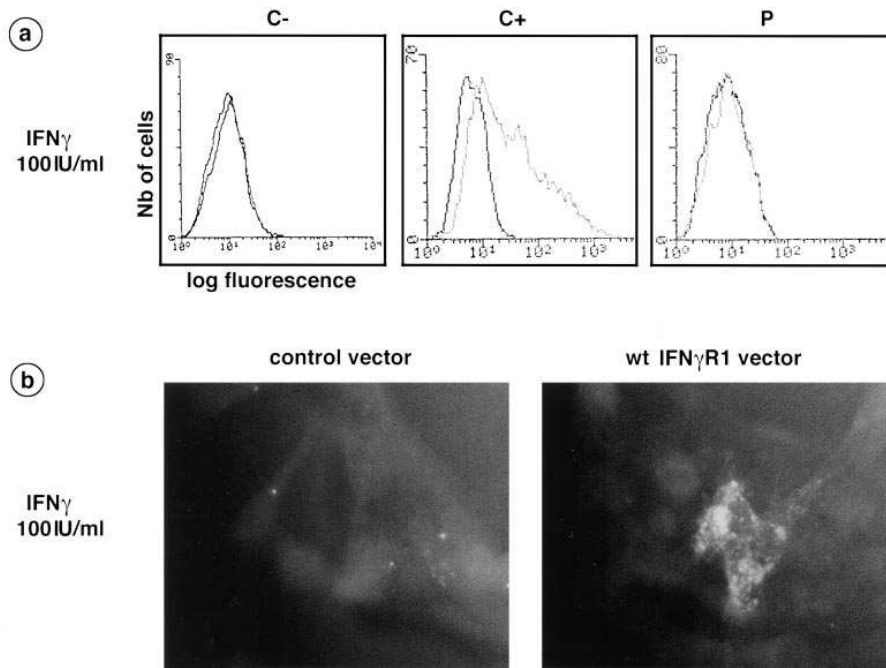


Figure 2. IFN γ R1 deficiency: impaired induction of HLA-II on fibroblasts in response to IFN- γ . (a) Flow cytometry analysis of HLA class II on transformed fibroblasts from a healthy individual (positive control, C+), a child with complete IFN γ R1 deficiency (negative control, C-), and a sibling (the girl) described herein (patient, P), with (gray line) or without (black line) IFN- γ (100 IU/ml). *Nb*, number. (b) Microscopic analysis of HLA class II induction on fibroblasts from the same patient as in a, transiently transfected with control expression vector or wild-type (wt) *IFNgR1* gene, in response to IFN- γ (100 IU/ml).

impaired IFN γ R1-mediated pathway, and suggesting that they had IFN γ R1 deficiency.

Sequencing of the *IFNgR1* mRNA coding region was then undertaken, and revealed a single mutation, a nucleotide substitution at position 260 (T \rightarrow C) leading to the replacement of an isoleucine by a threonine at position 87, which was designated as I87T (Fig. 3 a) (13). The mutation was confirmed at the genomic level in the third exon (14). The two siblings were found to be homozygous for the mutant I87T *IFNgR1* allele, and the two parents were found to be heterozygous (Fig. 3 b). The I87T *IFNgR1* allele has not been identified previously, neither in healthy individuals nor in children with complete IFN γ R1 deficiency (8). The identification of a homozygous missense mutation of the *IFNgR1* coding region further suggested that the two siblings had IFN γ R1 deficiency.

Transient transfection of the patient's fibroblasts with wild-type *IFNgR1* gene was able to restore a normal response to IFN- γ (100 IU/ml) (Fig. 2 b), strongly suggesting that the impaired response to IFN- γ was due to the I87T *IFNgR1* allele, and that the two children had IFN γ R1 deficiency (15). To ascertain the pathogenic role of this mutant allele, fibroblasts from a child with complete IFN γ R1 deficiency² were transiently transfected with wild-type or I87T *IFNgR1* gene, and the induction of HLA-II in response to IFN- γ (100 IU/ml) was assessed. Fibroblasts transfected with wild-type allele were able to respond to IFN- γ . However, those transfected with I87T allele failed to respond to IFN- γ (data not shown). These data demonstrated that the mutant I87T *IFNgR1* allele encoded a functionally deficient receptor, and that the two siblings had IFN γ R1 deficiency.

Partial, as opposed to complete, IFN γ R1 deficiency. The identification of a missense *IFNgR1* mutation in two siblings whose clinical and pathological features were less severe than those of children with complete IFN γ R1 deficiency suggested that the pathogenic IFN γ R1 molecules may be responsible for partial, as opposed to complete, IFN γ R1 deficiency. As a first approach, we analyzed cell surface expression of the receptor by

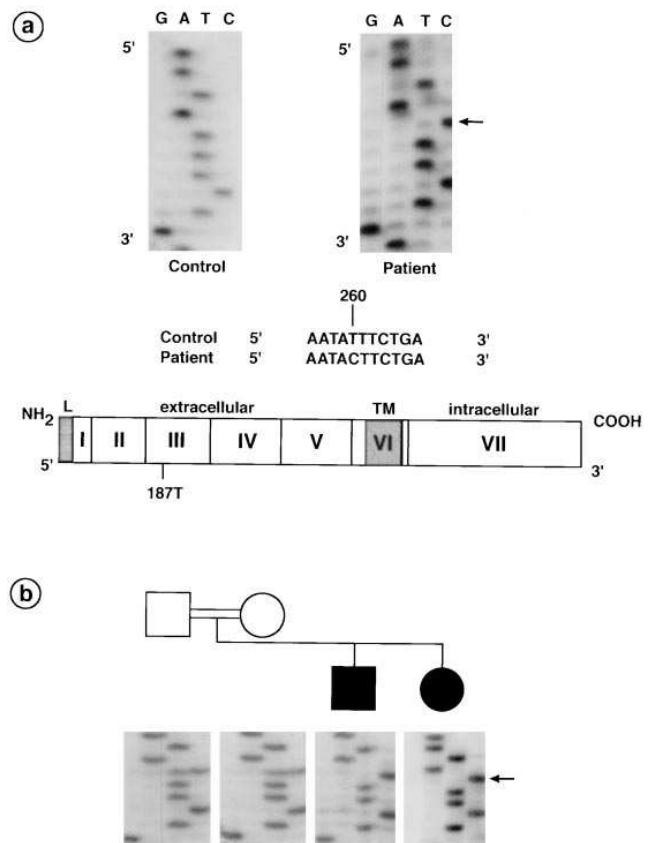


Figure 3. IFN γ R1 deficiency: a missense mutation of the *IFNgR1* coding region. (a) Sequence of the *IFNgR1* cDNA reveals a nucleotide substitution (T \rightarrow C) at position 260 (arrow), leading to the replacement of an isoleucine by a threonine at position 87, designated as I87T. (b) Intrafamilial segregation of nucleotides at position 260 (arrow), within the third genomic exon. The two siblings (filled symbols) are homozygous for the mutation (C/C), and the parents (open symbols) are heterozygous (C/T).

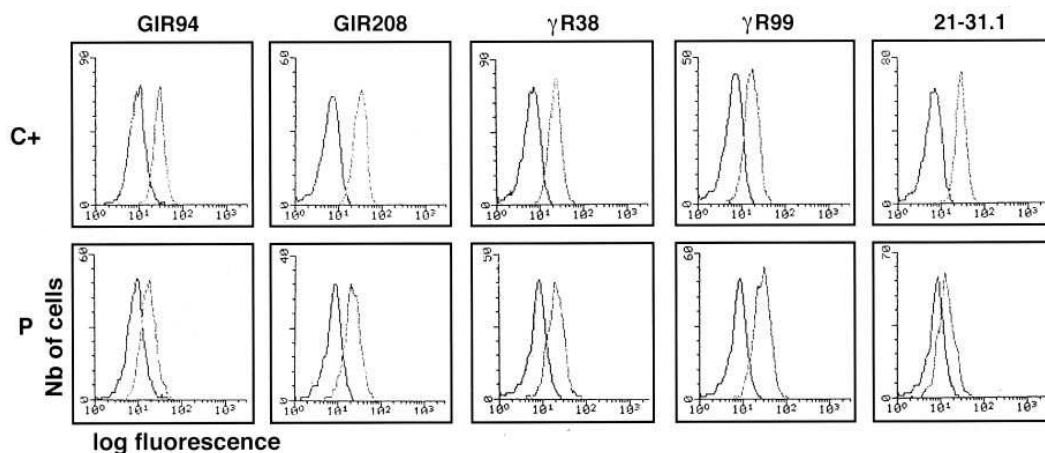


Figure 4. Partial IFN γ R1 deficiency: cell surface receptor expression. Cell surface detection of IFN γ R1 molecules by flow cytometry with five specific mAbs on monocytes from a sibling (the boy) (P), a child with complete IFN γ R1 deficiency (data not shown), and a control subject (C+). The background (black line) is that obtained with an isotypic control antibody. Nb, Number.

flow cytometry with five specific antibodies (11, 12). A positive staining with each of these antibodies was obtained on freshly isolated monocytes from each sibling (Fig. 4). Thus, the I87T *IFNgRI* mutation did not preclude cell surface expression of the encoded receptor, in contrast to the mutations identified previously in children with complete IFN γ R1 deficiency (1–8).² This further suggested that the defect in the two siblings was partial. To ascertain that the I87T *IFNgRI* allele was not a null allele despite normal receptor expression, and that the two siblings truly had partial deficiency, we attempted to show that I87T IFN γ R1 molecules could be functional in response to increasing IFN- γ concentrations.

Nuclear translocation of STAT1 homodimer is an early, specific, and easily detectable event after IFN- γ -mediated dimerization of functional IFN γ R1 molecules (16). Thus, we analyzed the IFN- γ -mediated nuclear translocation of STAT1 in EBV-transformed B cells by electrophoretic mobility shift assay (Fig. 5). In B cells from healthy individuals, nuclear translocation of STAT1 was observed at concentrations of IFN- γ ranging from 10 to 10,000 IU/ml. In contrast, B cells from a child with complete IFN γ R1 deficiency (4) showed no translocation of STAT1, even at high concentrations of IFN- γ . Transformed B cells from the two parents heterozygous for mutant I87T *IFNgRI* allele were found to respond normally to IFN- γ (data not shown). In contrast, B cells from the two siblings homozygous for mutant I87T *IFNgRI* allele failed to respond to low concentrations (10 and 100 IU/ml), as expected, yet responded to high concentrations of IFN- γ (10,000 IU/ml and, to a lesser extent, 1,000 IU/ml). This result strongly suggested that I87T IFN γ R1 molecules expressed on EBV-transformed B cells could be functional at high IFN- γ concentrations. To prove that I87T receptors could be functional in such conditions, fibroblasts from a child with complete IFN γ R1 deficiency² were transiently transfected with control vector, wild-type, or I87T *IFNgRI* allele, and the induction of HLA-II in response to low (100 IU/ml) or high IFN- γ concentrations (100,000 IU/ml) was assessed. Fibroblasts transfected with control vector were unable to respond to IFN- γ , and those transfected with wild-type allele were able to respond to IFN- γ at low and high concentrations. In contrast, fibroblasts transfected with I87T allele failed to respond to low, yet responded to high, IFN- γ concentrations (data not shown).

To ascertain that I87T IFN γ R1 molecules may also re-

spond to high IFN- γ concentrations in cell types more relevant to antimycobacterial immunity than EBV-transformed B cells and SV40-transformed fibroblasts, we investigated the effect of IFN- γ on freshly isolated monocytes. Monocytes and macrophages are the natural hosts and final killers of mycobacteria under physiological conditions, and much of the antimycobacterial effect of IFN- γ is believed to result from its macrophage-activating property. Thus, we tested the level of CD64 expression at the cell surface of freshly isolated monocytes in response to IFN- γ (Fig. 6A). Unlike monocytes from a child with complete IFN γ R1 deficiency, which did not respond to high concentrations of IFN- γ , and like monocytes from healthy individuals, monocytes from the two siblings were found to respond to high concentrations of IFN- γ . Comparable results were obtained when the level of TNF- α production by PBMCs (monocytes) in response to LPS and IFN- γ was quantified (5). In control individuals, the ratio of TNF- α produced in response to stimulation with LPS and IFN- γ (at concentrations ranging from 100 to 10,000 IU/ml) to TNF- α produced in response to stimulation with LPS alone was reproducibly measured between 2 and 2.5 (Fig. 6B). No increase in TNF- α production was obtained with cells from a child with complete IFN γ R1 deficiency, even at high IFN- γ concentrations (data not shown).

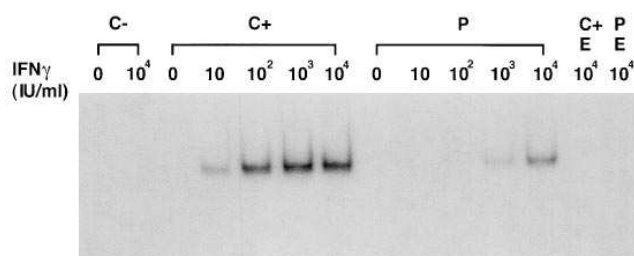


Figure 5. Partial IFN γ R1 deficiency: nuclear translocation of STAT1 in B cells. Nuclear translocation of STAT1 homodimers in response to IFN- γ , as detected by mobility shift assay in EBV-transformed B cells. Cells from a child with complete IFN γ R1 deficiency (C-), from a healthy individual (C+), and from one sibling (the boy) described herein (P), were activated by IFN- γ at concentrations ranging from 10 to 10,000 IU/ml. Competition with an excess of cold probe is shown in the two E lanes (right).

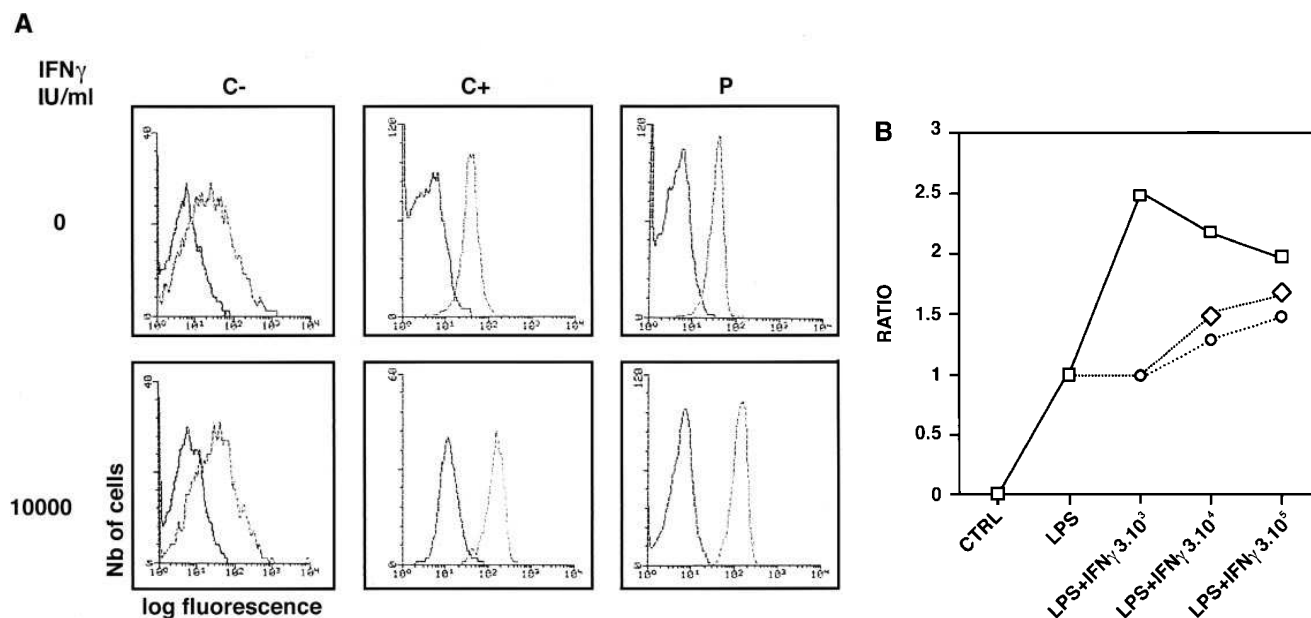


Figure 6. Partial IFN γ R1 deficiency: CD64 expression and TNF- α secretion by monocytes in response to high IFN- γ concentrations. (A) Cell surface induction of CD64 in response to IFN- γ , as detected by flow cytometry with specific antibodies on monocytes. Cells from a sibling reported herein (the boy) (P), a child with complete IFN γ R1 deficiency (C-), and a control subject (C+) were stimulated with 10,000 IU/ml IFN- γ and stained with a CD64-specific antibody. The background (black line) is that obtained with an isotypic control antibody. *Nb*, Number. (B) Production of TNF- α by PBMCs from the affected boy (diamonds and dotted line), the affected girl (circles and broken line), and a control individual (squares and solid line) in response to LPS alone or with IFN- γ at increasing concentrations (IU/ml).

In contrast, the two siblings showed a nearly normal response with high, albeit not with low or intermediate, IFN- γ concentrations (Fig. 6 B). Thus, the I87T *IFN γ R1* allele was responsible for an impaired, yet not completely abrogated, IFN- γ -mediated signaling in several cell types, including monocytes. A 100–1000-fold increase in IFN- γ concentration was able to trigger I87T IFN γ R1 molecules. Together, these data established that the two siblings suffered from partial IFN γ R1 deficiency.

Discussion

We have identified a missense I87T mutation of the *IFN γ R1* gene and shown that this mutant allele is responsible for partial, as opposed to complete, IFN γ R1 deficiency in two homozygous siblings. The isoleucine residue at position 87 (position 73 of the mature protein) is part of an N-glycosylation site in the first domain of the extracellular fragment of the receptor (17), at a position, however, that is theoretically permissive for most, if not all, amino acids (ASN-ILE-SER \rightarrow ASN-THR-SER). Biochemical consequences of this mutation are difficult to predict, since the substitution of isoleucine for threonine at position 87 may directly or indirectly impair (a) IFN γ R1 expression, (b) IFN γ R1 binding to IFN- γ , (c) dimerization of IFN γ R1, (d) IFN γ R1 association with IFN γ R2, and/or (e) IFN γ R1 coupling with intracellular cytosolic components such as Janus kinase 1 or STAT1 (16). Detection of cell surface molecules with specific antibodies suggested that the level of expression was not altered markedly. Nevertheless, the intensity of the signal varied from antibody to antibody (Fig. 4), when compared with control cells, perhaps suggesting that mi-

nor conformational changes were induced by the I87T substitution. Further studies are needed to unravel the biochemical basis of partially defective I87T IFN γ R1 molecules.

One affected child had disseminated BCG infection of favorable outcome with mature (paucibacillary, well-differentiated, and well-circumscribed) tuberculoid granulomas. This contrasts with the immature BCG (multibacillary, poorly differentiated, and poorly circumscribed) lepromatous-like granulomas and fatal outcome of a child reported previously with complete IFN γ R1 deficiency (4). Mice genetically deprived of *IFN γ* or *IFN γ R1* gene, referred to below as gamma knockout (gko) mice, were also shown to develop fatal BCG infection associated with lepromatous-like granulomas (18, 19). It is possible that IFN- γ concentrations obtained in vivo in children with partial IFN γ R1 deficiency are elevated sufficiently to promote mature granulomas. Nevertheless, the occurrence of disseminated BCG infection suggests that although the residual function of the receptor is sufficient for the generation of morphologically mature granulomas, the mycobactericidal properties of macrophages are not optimal. It is possible that the local concentrations of IFN- γ obtained in vivo do not elevate rapidly enough or sufficiently to trigger defective I87T receptors, thus facilitating the early stages of infection. In any event, there appears to be a correlation between genotype and phenotype, as the null *IFN γ R1* allele identified previously caused complete IFN γ R1 deficiency and fatal lepromatoid BCG infection, and the I87T allele, reported herein, caused partial IFN γ R1 deficiency and curable tuberculoid BCG infection.

Five children reported previously with complete IFN γ R1 deficiency developed disseminated NTM infection between 1

and 3 yr of age (5–8).² Comparable clinical features were observed recently in three other children from three newly identified kindreds with complete IFN γ R1 deficiency (our unpublished data). gko mice were also found to be susceptible to *M. avium* (20). In contrast, no NTM infection was diagnosed in the two siblings reported herein with partial deficiency who are currently 13 and 17 yr old. It may be that there is sufficient residual IFN- γ -mediated immunity to control environmental NTM, perhaps due to (a) the low virulence of most NTM species compared with BCG, or (b) the natural mode of infection with environmental NTM compared with inoculation with BCG vaccine. We have not yet investigated children with disseminated or localized NTM infection for partial IFN γ R1 deficiency. Nevertheless, together with the less severe BCG infection, the absence of NTM infection in this kindred suggests that the phenotype of children with partial IFN γ R1 deficiency is milder than that of children with complete deficiency. Other kindreds with complete and partial IFN γ R1 deficiency are needed to validate this hypothesis.

One sibling also suffered from disseminated non-typhi salmonella infection, attesting that salmonella infections may occur in IFN γ R1-deficient children, as suspected previously (2, 5). This confirms that IFN- γ plays an important role in the control of both mycobacteria and salmonella in healthy individuals. To our knowledge, salmonella infections in gko mice have not been reported. The occurrence in one sibling of a probable legionella infection suggests that IFN- γ may be important for the control of other opportunistic intracellular bacteria. gko mice were also found to be highly susceptible to legionella infection (21). Remarkably, as in previously reported children with complete IFN γ R1 deficiency (22), no other opportunistic infections have been documented in the two siblings with partial deficiency, and they responded normally to numerous common childhood viral and bacterial pathogens, contrasting with the apparently broader susceptibility of gko mice (22). It is uncertain whether partial IFN γ R1 deficiency in the two siblings predisposed them to clinical mycoplasma infection, as mycoplasma is a common pathogen in this age group, and the clinical course was unremarkable.

It is worthy of note that the sibling who was not inoculated with BCG presented with clinical primary tuberculosis. Given the susceptibility to mycobacterial infections associated with IFN γ R1 deficiency in other kindreds and the BCG infection in his sibling, it is likely that partial IFN γ R1 deficiency predisposed this child to clinical tuberculosis. Thus, partial IFN γ R1 deficiency appeared to be responsible for BCG and salmonella infections in one child and tuberculosis infection in the sibling, probably reflecting the different microbial exposures of the two siblings. It is probable that children with complete IFN γ R1 deficiency, exposed to tuberculous mycobacteria, would develop a more severe form of tuberculosis. gko mice die rapidly after infection with *M. tuberculosis* (23, 24). Susceptibility to tuberculosis is known to be associated with a number of inherited and acquired immunodeficiencies, such as AIDS. However, these conditions also predispose affected individuals to many severe infectious diseases other than tuberculosis. In this context, it is remarkable that the sibling with partial IFN γ R1 deficiency who suffered from clinical tuberculosis did not suffer from any other unusual infections.

In keeping with the observation that only a minority of individuals infected with *M. tuberculosis* develop clinical disease (25), it is tempting to speculate that clinical tuberculosis in oth-

erwise healthy individuals in the general population may be associated with partial IFN γ R1 deficiency. The recognition of a familial defect due to a mutation producing partial IFN γ R1 deficiency which has resulted in susceptibility to both BCG infection and tuberculous infection provides the first evidence that defects in this gene may play a role in tuberculosis susceptibility. In the short time period since the first reports of IFN γ R1 deficiency, a range of different mutations in this gene have now been identified, from those causing complete absence of expression to those causing subtle alterations in the function of the receptor (references 1–8, this report, and our unpublished data).² Thus, it is tempting to speculate that homozygous or compound heterozygous carriers of mutations causing mild functional impairment of the receptor may be prevalent in different ethnic populations, and may help to explain susceptibility to tuberculosis within the general population.

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References

1. Casanova, J.-L., E. Jouanguy, S. Lamhamedi, S. Blanche, and A. Fischer. 1995. Immunological conditions of children with BCG disseminated infection. *Lancet*. 346:581.
2. Casanova, J.-L., S. Blanche, J.-F. Emile, E. Jouanguy, S. Lamhamedi, F. Altare, J.-L. Stephan, F. Bernaudin, P. Bordigoni, D. Turck, et al. 1996. Idiopathic disseminated bacillus Calmette-Guérin infection: a French national retrospective study. *Pediatrics*. 98:774–778.
3. Emile, J.-F., N. Patey, F. Altare, S. Lamhamedi, E. Jouanguy, F. Boman, J. Quillard, M. Lecomte-Houcke, O. Verola, J.-F. Mousnier, et al. 1997. Correlation of granuloma structure with clinical outcome defines two types of idiopathic disseminated BCG infections. *J. Pathol.* 181:25–30.
4. 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- γ receptor deficiency in an infant with fatal bacille Calmette-Guérin infection. *N. Engl. J. Med.* 335:1956–1961.
5. Levin, M., M. Newport, S. D'Souza, P. Kalabalibis, I.N. Brown, H.M. Lenicker, P. Vassallo-Agius, E.G., Davies, A. Trasher, N. Klein, and J.M. Blackwell. 1995. Familial disseminated atypical mycobacterial infection in childhood: a human mycobacterial susceptibility gene? *Lancet*. 345:79–83.
6. Newport, M.J., C. Huxley, S. Huston, C.M. Hawrylowicz, B.A. Oostea, R. Williamson, and M. Levin. 1996. A mutation in the interferon- γ receptor gene and susceptibility to mycobacterial infection. *N. Engl. J. Med.* 335:1941–1949.
7. Pierre-Audigier, C., E. Jouanguy, S. Lamhamedi, F. Altare, J. Raugier, V. Vincent, D. Canioni, J.-F. Emile, A. Fischer, S. Blanche, et al. 1997. Fatal disseminated *Mycobacterium smegmatis* infection in a child with inherited interferon- γ receptor deficiency. *Clin. Infect. Dis.* 24:982–984.
8. Casanova, J.-L., M. Newport, A. Fischer, and M. Levin. 1997. Inherited interferon γ receptor deficiency. In *Primary Immunodeficiencies, a Molecular and Genetic Approach*. J. Puck, H. Ochs, and C. Smith, editors. Oxford University Press, New York. In press.
9. 1995. Report of a WHO Scientific Group. Primary immunodeficiency diseases. *Clin. Exp. Immunol.* 99:1–24.
10. Greenlund, A.C., M.A. Farrar, B.L. Viviano, and R.D. Schreiber. 1994. Ligand-induced IFN γ receptor tyrosine phosphorylation couples the receptor to its signal transduction system (p91). *EMBO (Eur. Mol. Biol. Organ.) J.* 13: 1591–1600.
11. Garotta, G., L. Ozmen, M. Fountoulakis, Z. Dembic, A.P.G.M. van Loon, and D. Stüber. 1990. Human interferon- γ receptor. Mapping of epitopes

recognized by neutralizing antibodies using native and recombinant receptor proteins. *J. Biol. Chem.* 265:6908–6915.

12. Sheehan, K.C.F., J. Calderon, and R.D. Schreiber. 1988. Generation and characterization of monoclonal antibodies specific for the human IFN γ receptor. *J. Immunol.* 140:4231–4237.

13. Aguet, M., Z. Dembic, and G. Merlin. 1988. Molecular cloning and expression of the human IFN γ receptor. *Cell.* 55:273–280.

14. Merlin, G., B.J. van der Leede, K. McKune, N. Knezevic, W. Bannwarth, N. Romquin, E. Viegas-Pequignot, H. Kiefer, M. Aguet, and Z. Dembic. 1997. The gene for the ligand binding chain of the human interferon gamma receptor. *Immunogenetics.* 45:413–421.

15. Altare, F., E. Jouanguy, M. Newport, S. Lamhamedi, A. Fischer, M. Levin, and J.-L. Casanova. 1997. *IFNGR1*, a human mycobacterial susceptibility candidate gene. *Bull. Inst. Pasteur/Res. Infect. Dis.* 95:143–146.

16. Bach, E.A., M. Aguet, and R.D. Schreiber. 1997. The gamma interferon receptor, a paradigm for cytokine receptor signaling. *Annu. Rev. Immunol.* 15: 563–592.

17. Walter, M.R., W.T. Windsor, T.L. Nagabhushan, D.J. Lundell, C.A. Lunn, P.J. Zauodny, and S.K. Narula. 1995. Crystal structure of a complex between interferon γ and its soluble high-affinity receptor. *Nature.* 376:230–235.

18. 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- γ genes. *Science.* 259:1739–1742.

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

20. Doherty, T.M., and A. Sher. 1997. Defects in cell-mediated immunity affect chronic, but not innate, resistance of mice to *Mycobacterium avium* infection. *J. Immunol.* 158:4822–4831.

21. Heath, L., C. Chrisp, G. Huffnagle, M. Legendre, Y. Osawa, M. Hurley, C. Engleberg, J. Fantone, and J. Brieland. 1996. Effector mechanisms responsible for γ 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.

22. Jouanguy, E., F. Altare, S. Lamhamedi, and J.-L. Casanova. 1997. Infections in IFN γ R1-deficient children. *J. Interferon Cytokine. Res.* 17:583–587.

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

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

25. Stead, W.W. 1992. Genetics and resistance to tuberculosis. *Ann. Intern. Med.* 116:937–941.