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Survey

# Interferon-γ and interleukin-12 pathway defects and human disease

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**Abstract**

A genetic component to human mycobacterial disease susceptibility has long been postulated. Over the past five years, mutations in the interferon-γ (IFNγ) receptor, IL-12 receptor β1 (IL-12Rβ1), and IL-12 p40 genes have been recognized. These mutations are associated with heightened susceptibility to disease caused by intracellular pathogens including nontuberculous mycobacteria, vaccine-associated bacille Calmette Guerin (BCG), *Salmonella* species, and some viruses. We describe the genotype-phenotype correlations in IFNγ receptor, IL-12Rβ1, and IL-12 p40 deficiency, and discuss how study of these diseases has enhanced knowledge of human host defense against mycobacteria and other intracellular pathogens. Published by Elsevier Science Ltd.

*Keywords:* Interferon-gamma; Interferon-gamma receptor deficiency; Interleukin-12; Tuberculosis; Mycobacteria

**Contents**



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## 1. Introduction

Infections with intracellular bacteria such as mycobacteria remain an important cause of human morbidity and mortality worldwide. Immunologic protection against such organisms depends on cell mediated immunity, the major effector of which is the IFNγ-activated macrophage. The importance of IFNγ pathways in host defense against intracellular bacteria was initially made clear through the experimental study of knockout mice. More recently, the identification and characterization of humans with mutations in IFNγ receptor proteins, IL-12 receptor β1, or IL-12 p40 has confirmed the importance of these pathways in human host defense.

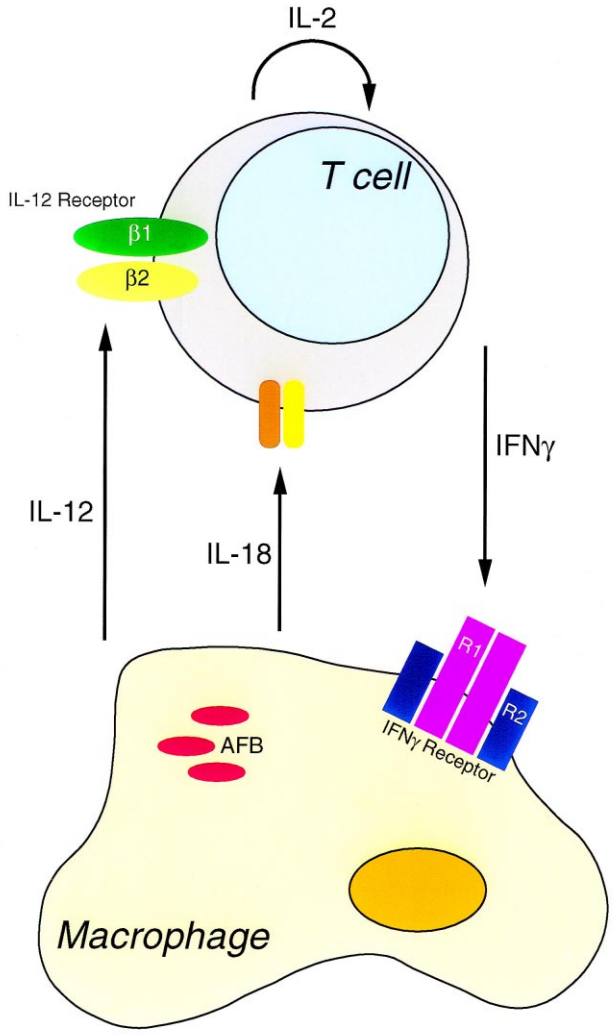


Fig. 1. IFNγ and IL-12 production and response pathways. In response to infection with mycobacteria, macrophages produce cytokines including IL-12. IL-12 and IL-18 synergistically stimulate production of IFNγ by $CD4^+$ T cells and NK cells. IFNγ ligand binds to its receptor on the macrophage cell surface and activates the macrophage resulting in enhanced TNFα production, production of cytokines and chemokines, upregulation of MHC class II expression, enhanced antigen processing, and production of reactive oxygen and (in mice) nitrogen intermediates.

## 2. IFNγ and the IFNγ receptor

IFNγ was first identified on the basis of its in vitro antiviral activity [1]. It is produced predominantly by T cells and NK cells in response to a variety of inflammatory or immune stimuli, and in general, it stimulates the development and function of immune effector cells. IL-12 and IL-18 secreted by macrophages and dendritic cells are thought to be the primary inducers of IFNγ production in an inflammatory reaction [2–5] (Fig. 1).

IFNγ receptors are expressed on almost all nucleated cells, and show species specificity in their ability to bind IFNγ [6]. The functional IFNγ receptor is composed of two 90 kDa IFNγR1 (formerly α or ligand-binding chain, or CD119w) proteins and two 62 kDa IFNγR2 (formerly β or signal transducing chain, or accessory factor-1) proteins [7]. The human IFNGR1 gene contains seven exons, and is located on chromosome 6 [8]. The extracellular portion of IFNγR1 contains the IFNγ ligand-binding domain; the intracellular portion contains domains necessary for signal transduction and receptor recycling [6,7] (Fig. 2). The IFNGR2 gene also contains seven exons, and is located on human chromosome 21 [9,10]. The extracellular domain of IFNγR2 interacts with the IFNγR1/IFNγ complex, but does not itself play a major role in ligand binding [7]. The intracellular IFNγR2 domain is necessary for signal transduction [11] (Fig. 2). In the absence of stimulation, IFNγR1 and IFNγR2 are not strongly associated with each other. However, inactive Janus kinase 1 (JAK1) is bound to the four amino acid sequence ($_{266}$LPKS$_{269}$) in the membrane proximal IFNγR1 intracellular domain [7], and inactive JAK2 is bound to a proline-

rich sequence ($_{263}$PPSIPLQIEEYL$_{274}$) in the IFNγR2 intracellular domain [12].

IFNγ binds as a homodimer to two IFNγR1 proteins, thereby facilitating the binding of two IFNγR2 proteins to the IFNγR1/IFNγ complex [12–16] (Fig. 3). Within this complex the IFNγR1 and IFNγR2 intracellular domains, with their constitutively associated JAKs, are brought into proximity. Ligand binding results in reciprocal transphosphorylation of the JAKs, and subsequent phosphorylation of IFNγR1 $Y_{440}$ [7,17]. Through its SH2 domain, one latent STAT1 recognizes and binds to each tyrosine phosphorylated IFNγR1 ($_{440}$YDKPH$_{444}$) site [18]. Receptor associated STAT1 proteins are subsequently tyrosine phosphorylated and, so activated, form homodimers that translocate to the nucleus where they bind to IFNγ activation sequences (GAS) of IFNγ-inducible genes [19–23]. The intracellular IFNγR1 motif $_{270}$LI$_{271}$ is important for directing receptor trafficking through the cell, including recycling of the receptor off of the cell surface after ligand binding [6,24].

Three observations indicate a biological role for intracellular IFNγ. First, IFNγ delivered by liposomes has been shown to activate murine macrophages to a tumoricidal state [25]. Second, microinjected IFNγ can induce Ia expression on murine macrophages [26]. Finally, secretion-defective human IFNγ expressed in murine fibroblasts induces an antiviral state in those cells [27]. Recently it was demonstrated that a polybasic nuclear localization sequence in the carboxyl terminus of IFNγ is required for nuclear translocation and biological activity of this cytokine [28]. Both human and murine IFNγ have been shown to interact with the cytoplasmic domain of their species-matched IFNγR1. A plausible role for IFNγ is that of an intracellular chaperone that facilitates nuclear translocation of STAT1 which itself lacks a nuclear translocation sequence [28,29].

IFNγ activates transcription of a large number of genes that play roles in antiviral activity, apoptosis, antigen processing, MHC protein expression, and type 1 T helper cell (TH1) development. IFNγ also activates macrophages to kill or restrict growth of microbial targets; this function appears to be important in host defense against mycobacteria. In mice, IFNγ-induced generation of reactive nitrogen intermediates is one mechanism of *Mycoplasma tuberculosis* killing [30,31].

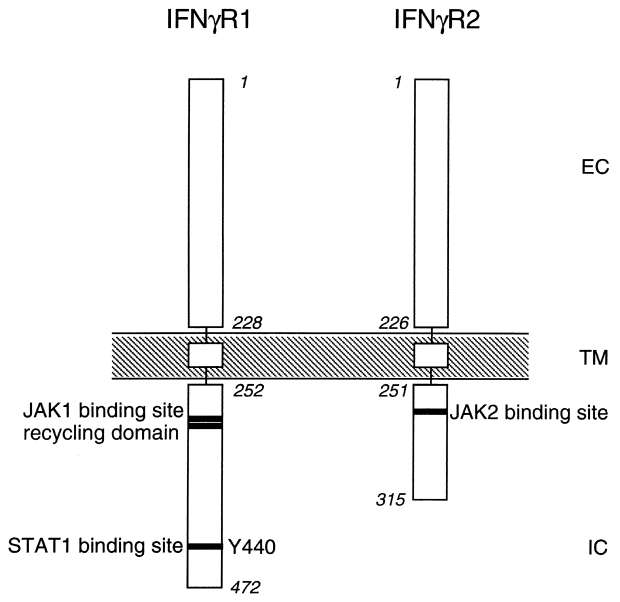


Fig. 2. Schematic representation of the human IFNγ receptor 1 (IFNγR1), and the human IFNγ receptor 2 (IFNγR2). Functionally important intracellular domains are identified, and numeric positions of amino acids are shown. STAT: signal transducer and activator of transcription; JAK: Janus kinase.

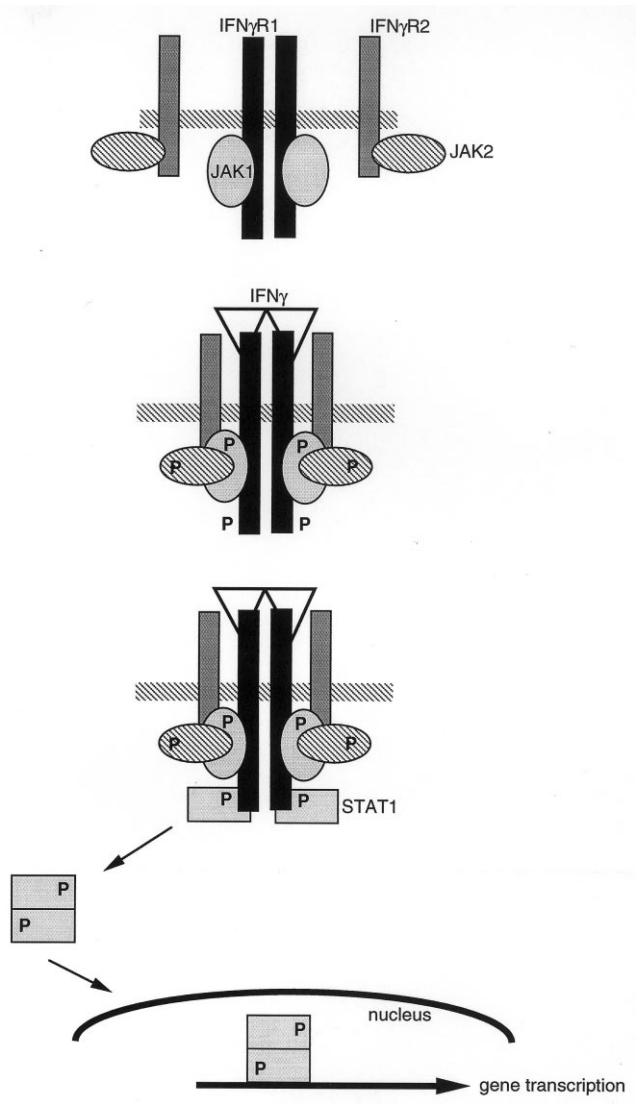


Fig. 3. Schematic representation of the human IFNγ signal transduction pathways. See text for details.

However, reactive nitrogen intermediates have not been shown conclusively to play a major role in mycobacterial killing by human cells. Despite our understanding of IFNγ signal transduction, the pathways by which this cytokine activates mycobactericidal macrophage activities in humans are poorly understood.

## 3. IL-12 and the IL-12 receptor

IL-12 is a heterodimeric cytokine produced primarily by antigen presenting cells. It enhances proliferation and cytolytic activity of natural killer (NK) and T cells, and stimulates their IFNγ production [32]. IL-12 plays a key role in promoting TH1 responses and subsequent cell mediated immunity [33–35]. Production of IL-12 stimulated by microbial lipoproteins, including a 19-kD *M. tuberculosis* lipoprotein, is mediated by Toll-like receptors [36]. IL-12 is composed of two disulfide-linked subunits, p35 and p40, which are encoded by unrelated genes on human chromosomes 3 and 5, respectively [37–39]. Functional IL-12 receptors are expressed primarily on activated T and NK cells [40]. Two IL-12 receptor subunits, IL-12Rβ1 and IL-12Rβ2, have been cloned from human and mouse T cells [41–43]. Coexpression of these two subunits is required for high affinity binding of IL-12 [43]. Expression of IL-12Rβ2 is tightly controlled and may be an important mechanism for regulation of IL-12 responsiveness [44].

## 4. Knock-out murine models

The importance of IFNγ pathways in host defense has been demonstrated in mice with targeted disruptions of the IFNγ, IFNGR1, or IFNGR2 genes [45]. After experimental inoculation, IFNγ and IFNGR1 knockout mice have increased susceptibility to experimental challenge with a wide spectrum of infectious agents, including mycobacteria [46–49], bacteria [50,51], parasites [52–54], and viruses [55–58]. In contrast to wild-type (WT) mice, IFNγ and IFNGR1 knockout mice develop neither mature granulomas nor protective immunity after experimental infection with *Mycobacterium bovis* bacille Calmette-Guerin (BCG) [48,49]; IFNγ knockout mice develop neither mature granulomas nor protective immunity after experimental infection with *M. tuberculosis* Erdman strain [46,47]. IFNγ knockout mice experimentally infected with certain *Mycobacterium avium* strains develop higher tissue levels of bacteria than WT mice [59], and they do not develop protective immunity to some attenuated strains of *Salmonella typhimurium* [60,61]. Of note, neither IFNGR1 nor IFNγ knockout mice develop spontaneous infection with environmental nontuberculous mycobacteria (NTM), even when housed in non-sterile facilities [62]. Mice with targeted disruptions of the IFNGR2 gene have been less well characterized, but have been shown to be highly susceptible to sublethal challenge with *Listeria monocytogenes* [63].

Direct comparison between IFNγ and IFNGR1 knockout mice has been problematic because the mouse strains have had different genetic backgrounds. Recently the responses to HSV1 or vaccinia virus challenges were compared in IFNγ and IFNGR1 knockout mice derived from the same genetic background [64]. Mortality from challenge with either virus was significantly greater in IFNGR1 knockout mice than IFNγ knockout mice. The mechanism underlying these differences has not been established, and it is not yet known if results of mycobacterial challenge would be different in IFNGR1 versus IFNγ knockout mice.

Mice with disrupted genes for IL-12 p40 or IL-12Rβ1 have also been described. Compared with WT mice, IL-12 p40 knockout mice are more susceptible to experimental infection with BCG [65,66], *M. tuberculosis* [67], and virulent *M. avium* [68], and they fail to form mature granulomas in response to BCG and *M. tuberculosis* [65–67]. It appears that IFNγ or IFNGR1 knockout mice are more susceptible than IL-12 p40 or IL-12Rβ1 knockout mice to experimental infection with BCG and *M. tuberculosis*, but no study has directly compared the mycobacterial susceptibilities of these knockout models. Overall, knockout mice are good models for studying some aspects of mycobacterial immunity and pathogenesis. However, differences in the role of reactive nitrogen intermediates [30,31], and in manifestations of mycobacterial infection (e.g. experimental tuberculosis in wild-type mice is a chronic, ultimately fatal pulmonary disease) somewhat limit the extrapolation to humans of results obtained in knockout mice.

## 5. Human IFNγ receptor deficiencies

The existence of a genetic component to human mycobacterial disease susceptibility has long been postulated. Differences in susceptibility to *M. tuberculosis* infection among different racial groups [69] and in twins [70], and manifestations of leprosy [71] support this hypothesis. Also in support of this idea is a tragic incident in which a single virulent viable *M. tuberculosis* strain was inadvertently used to immunize infants [72,73]. Responses to the vaccine ranged from death to recovery, arguing for a genetic basis for resistance to tuberculosis.

Elucidation of the critical role of IFNγ receptor genes in control of nontuberculous mycobacterial (NTM) infection began with the identification of kin-

dreds in whom affected individuals had severe infection with poorly virulent environmental mycobacteria, in the absence of a known immunodeficiency [74–76]. Parental consanguinity in some of these kindreds suggested a Mendelian disorder of autosomal recessive inheritance [74]. Immunologic investigation of four related Maltese children who had disseminated NTM infections showed diminished TNFα production in response to stimulation with IFNγ plus endotoxin in a whole blood assay [77]. A subsequent genome-wide search using microsatellite analysis identified a region on chromosome 6q for which all affected children in this family were homozygous [78]. IFNGR1 was known to map to that chromosomal region, and was further investigated. Patient leukocytes lacked expression of IFNγR1 protein, and DNA sequencing of the IFNGR1 gene revealed the affected patients to be homozygous for a point mutation resulting in creation of a premature termination codon. The simultaneous report of an infant with disseminated vaccine-associated BCG infection and a different chain terminating mutation in IFNGR1 [79] firmly established the importance of IFNγ responsiveness in control of both vaccine-associated BCG infection and environmentally-acquired NTM infections. Subsequently we identified a child with disseminated *M. fortuitum* and *M. avium* complex (MAC) infections, in whom genetic analysis showed an IFNGR2 frameshift mutation which created a premature stop codon and was associated with complete absence of IFNγ responsiveness [80]. IFNγ receptor mutations have since been described in individuals from many parts of the world and many ethnic groups (Table 1). Missense mutations, small inframe deletions or insertions, nonsense or frameshift mutations resulting in creation of a premature stop codon, and aberrant splicing events resulting in larger deletions have been described. Phenotype-to-genotype correlations are being established as more affected individuals are identified. For IFNγ receptor deficiency, the phenotype appears to depend less on which gene (IFNGR1 vs IFNGR2) is mutated, but rather on the extent to which the mutation reduces IFNγ responsiveness.

### 5.1. Complete IFNγ receptor deficiency

Complete absence of IFNγ responsiveness due to a mutation in either IFNGR1 or IFNGR2 is associated with a severe clinical phenotype. Such affected individuals characteristically have severe disseminated mycobacterial infections that may involve lungs, viscera, lymph nodes, blood, and bone marrow. Onset of first environmentally acquired mycobacterial infection is usually during infancy. Infections are typically caused either by NTM species that are poorly pathogenic in immunocompetent hosts and presumably acquired from environmental exposure, or by BCG acquired by vaccination. In these children, such infections are usually fatal if untreated. Aggressive and prolonged antibiotic therapy can lead to control of infection in some patients. However, the overall prognosis for these patients is poor since antibiotic therapy apparently does not completely eradicate organisms, and there is continued susceptibility to new mycobacterial infection. Based on the current understanding of IFNγ signal transduction, IFNγ administration would not be expected to be of therapeutic benefit in patients with complete absence of IFNγ responsiveness in vitro. In a small number of patients, bone marrow transplantation has been effective in curing the genetic defect in hematopoeitic cells, and eliminating the phenotype of heightened mycobacterial infection susceptibility ([85], JL Casanova, personal communication).

Histologic examination of mycobacteria-infected tissues from patients with complete IFNγR1 or complete IFNγR2 deficiency typically shows granulomas which are poorly circumscribed, poorly differentiated, and multibacillary (lepromatoid), implying that IFNγ is required for mature granuloma formation in the setting of mycobacterial infection [79,80,93]. However, tuberculin-specific delayed-type hypersensitivity (DTH) responses are typically normal in *M. bovis* BCG-infected children with complete IFNγR1 deficiency, implying that IFNγ is not necessary for development of DTH responses in humans. In vitro, PBMC from patients with complete IFNγ receptor deficiency produce low amounts of IFNγ and IL-12 in response to phytohemagglutinin (PHA), indicating that IFNγ plays a role in regulation of itself and IL-12 [81]. In the two identified patients with complete IFNγR2 deficiency, the clinical features, histopathology, and results of in vitro functional studies are the same as in patients with complete IFNγR1 deficiency [80,86].

Heterozygous parents and siblings of children with autosomal recessive complete IFNγ receptor deficiency do not appear to have increased susceptibility to mycobacterial infections, although the number of such individuals studied is small and none have been studied in tuberculosis endemic areas. We have found that PBMC from these heterozygous relatives have normal in vitro IFNγ responsiveness, as measured by IFNγ-stimulated TNFα production [81]. Therefore, haploinsufficiency does not appear to be associated with an abnormal clinical or in vitro functional phenotype. However, in vitro challenge of these cells with a biologic stimulus such as *M. tuberculosis* has not been performed.

### 5.2. Dominant negative partial IFNγR1 deficiency

Dominant negative effects have been shown conclusively to result from one group of IFNGR1 mutations

Table 1
Patients with mycobacterial infections and defined gene mutations[a]

| Defect | Patient | Ethnicity | Infections | Zygosity | Mutation | Ref. |
|---|---|---|---|---|---|---|
| c-IFNγR1 | 1 | Maltese | *M. avium* | Homozygous | S116X | [78] |
| | 2 | Maltese | *M. avium*; *Salmonella* | Homozygous | S116X | [78] |
| | 3 | Maltese | *M. chelonei* | Homozygous | S116X | [78] |
| | 4 | Maltese | *M. fortuitum* | Homozygous | S116X | [78] |
| | 5 | Tunisian | BCG | Homozygous | 131delC | [79] |
| | 6 | Pakistani | *M. avium* | Homozygous | 22delC | [81,82] |
| | 7 | Pakistani | *M. avium* | Homozygous | 201–2 A → G | [81] |
| | 8 | Italian | *M. smegmatis* | Heterozygous | 107ins4; 200 + 1 G → A | [83,84] |
| | 9 | Italian | BCG? | Heterozygous | 107ins4; 200 + 1 G → A | [83,84] |
| | 10 | Italian | BCG? | Heterozygous | 107ins4; 200 + 1 G → A | [83,84] |
| | 11 | Italian | BCG? | Heterozygous | 107ins4; 200 + 1 G → A | [83,84] |
| | 12 | German | BCG; *M. avium*; *M. kansasii*; *L. monocytogenes* | Heterozygous | 561del4; 373 + 1 G → T | [85] |
| | 13 | Argentinian | BCG | Homozygous | 561del4 | Unpublished |
| c-IFNγR2 | 14 | English/Portuguese | *M. avium*; *M. fortuitum* | Homozygous | 278delA,G | [80] |
| | 15 | Qatari | BCG? *M. abscessus* | Homozygous | 791delG | [86] |
| AR p-IFNγR1 | 16 | Portuguese | BCG; *S. enteritidis*; *L. pneumophila*? | Homozygous | I87 T | [87] |
| | 17 | Portuguese | *M. tuberculosis*? | Homozygous | I87 T | [87] |
| AR p-IFNγR2 | 18 | Portuguese | BCG; *M. abscessus* | Homozygous | R114C | [88] |
| AD p-IFNγR1 | 19 | Irish | *M. avium; M. spp.* | Heterozygous | 818del4 | [89] |
| | 20 | Irish | *M. avium* | Heterozygous | 818del4 | [89] |
| | 21 | Irish | *M. avium* | Heterozygous | 818del4 | [89] |
| | 22 | Irish | BCG; *M. avium* | Heterozygous | 818del4 | [89] |
| | 23 | Irish | BCG | Heterozygous | 818del4 | [89] |
| | 24 | German | BCG; *M. spp.* | Heterozygous | 818del4 | [89] |
| | 25 | German | BCG | Heterozygous | 818del4 | [89] |
| | 26 | Moroccan | BCG; *M. avium*; *M. kansasii* | Heterozygous | 818del4 | [89] |
| | 27 | Swedish | *M. avium* | Heterozygous | 818del4 | [89] |
| | 28 | English | *M. avium* | Heterozygous | 818del4 | [89] |
| | 29 | American | *M. avium* | Heterozygous | 818del4 | [89] |
| | 30 | American | *M. avium* | Heterozygous | 818del4 | [89] |
| | 31 | American | *M. avium* | Heterozygous | 818del4 | [89] |
| | 32 | American | *M. avium*; *H. capsulatum* | Heterozygous | 818del4 | [89] |
| | 33 | American | *M. avium* | Heterozygous | 818del4 | [89] |
| | 34 | American | *M. avium* | Heterozygous | 818del4 | [89] |
| | 35 | Scottish | BCG | Heterozygous | 818del4 | [89] |
| | 36 | Italian | BCG; *M. avium* | Heterozygous | 818delT | [89] |
| | 37 | Korean/African | *M. avium; M. kansasii*; *M. chelonei* | Heterozygous | 817insA | Unpublished |
| c-IL-12 p40 | 38 | Pakistani | BCG; *S. enteritidis* | Homozygous | p40del4.4 | [90] |
| c-IL-12Rβ1 | 39 | Turkish | BCG; *S. typhimurium* | Homozygous | del409-549 | [91] |

| | | | | | |
|---|---|---|---|---|---|
| 40 | Dutch | *M. avium; S. paratyphi* | Homozygous | Q32X | [91] |
| 41 | Dutch | *M. avium; S. group B* | Homozygous | Q376X | [91] |
| 42 | Moroccan | BCG; *S. enteritidis* | Homozygous | K305X | [92] |
| 43 | Turkish | BCG | Homozygous | 783+1 G→C | [92] |
| 44 | Cypriot | *M. avium; S. enteritidis* | Homozygous | Q214R | [92] |
| 45 | Cypriot | *M. avium* | Not identified[b] | | [92] |

[a] Abbreviations: BCG, bacille Calmette Guerin; c-IFNγR1, complete interferon gamma receptor 1 deficiency; AD p-IFNγR1, autosomal dominant partial interferon gamma receptor 1 deficiency; AR p-IFNγR1, autosomal recessive partial interferon gamma receptor 1 deficiency; c-IFNγR2, complete interferon gamma receptor 2 deficiency; p-IFNγR2, partial interferon gamma receptor 2 deficiency; c-IL-12p40, complete IL-12 p40 deficiency; c-IL-12Rβ1, complete IL-12 receptor β1 deficiency.
[b] Deceased brother of patient 44; mutation not identified but likely to be Q214R.

[89]. These mutations result in a premature stop codon in the proximal intracellular protein domain, and they confer partial, but not complete, loss of IFNγ responsiveness. The IFNGR1 mutations with autosomal dominant effects are 818del4 and 818delT [89], and 817insA (data not shown). IFNGR1 818del4 is the most common, occurring in at least 11 unrelated kindreds. Surrounding nucleotide analysis supports a model of slipped mispairing during replication as the mechanism causing 818del4 mutations [89,94]. Mutant proteins are expressed on the cell surface and bind IFNγ ligand, but cannot transduce signal due to absence of JAK1 and STAT1 binding sites (Fig. 4A). Moreover, the absence of the IFNγR1 recycling motif results in an increased number of mutant proteins expressed on the cell surface (Fig. 4B). Residual IFNγ responsiveness is mediated by the normal IFNγR1 proteins expressed from the normal allele in these heterozygous individuals. PBMC TNFα production in response to IFNγ plus lipopolysaccharide is approximately three-fold lower in patients with autosomal dominant IFNGR1 818del4 mutations than in normals (Dorman and Holland, unpublished data).

The clinical phenotype associated with this group of autosomal dominant (AD) mutations is milder than that seen in children with complete absence of IFNγ responsiveness. Environmental mycobacterial infec-

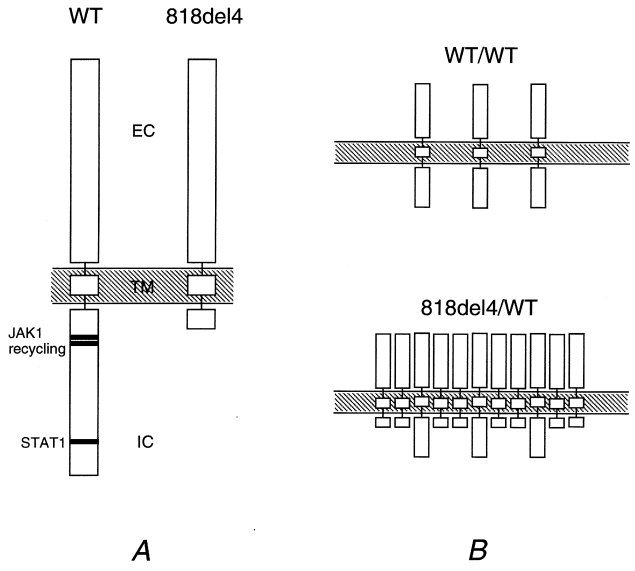


Fig. 4. Schematic representation of the dominant negative IFNGR1 818del4-encoded protein. (A) The mature wild-type (WT) and 818del4 IFNγR1 proteins, with extracellular (EC), transmembrane (TM), intracellular (IC), JAK1 binding, receptor recycling, and STAT1 binding sites shown. The 818del4 protein lacks the latter three intracellular motifs. (B) Schematic representation of IFNγ receptors at the surface of cells homozygous for the wild-type allele (WT/WT, top), or heterozygous (818del/WT, bottom). Adapted from Ref. [89].

tions may first occur during childhood rather than infancy, may be localized rather than disseminated, and are usually responsive to appropriate antimicrobial therapy. Granulomas are usually paucibacillary and mature ([89]). Interestingly, we have observed that the majority of patients with IFNGR1 818del4 mutations develop multifocal NTM osteomyelitis, often without infection at other sites. The pathophysiologic basis for this is unclear. Anecdotal evidence supports the adjunctive use of IFNγ therapy in patients with AD partial IFNγR1 deficiency (Dorman and Holland, unpublished data). However, controlled studies of IFNγ therapy during episodes of active infection, or IFNγ prophylaxis to prevent infections have not yet been performed.

### *5.3. Autosomal recessive partial IFNγR1 and IFNγR2 deficiencies*

Two siblings with autosomal recessive (AR) partial IFNγR1 deficiency have been described [87]. At age 1 month, the elder child developed disseminated vaccine-associated BCG infection which was associated with mature granulomas on histopathologic examination of affected tissue, and which responded well to antibiotic therapy. The younger sibling did not receive BCG vaccination, but at age 3 years she developed an illness compatible with primary tuberculosis that responded well to antituberculous therapy. IFNGR1 gene sequencing revealed both children to be homozygous for a single nucleotide substitution leading to replacement of an isoleucine by a threonine at position 87 (I87T) that is part of an N-glycosylation site in the extracellular protein domain. Constructs of this mutation were associated with diminished but not absent responsiveness to IFNγ in vitro. The degree of IFNγ responsiveness as measured by nuclear translocation of STAT1 in EBV-transformed B lymphocytes from these siblings was greater than that from children with AD partial IFNGR1 deficiency. This may account for the absence of environmentally acquired NTM infections in the siblings with AR partial IFNγR1 deficiency. If so, then subtle alterations in IFNγ mediated pathways may allow the development of disease due to virulent mycobacteria like *M. tuberculosis* but protect against disease caused by less virulent environmental NTM.

Recently a patient with autosomal recessive partial IFNγR2 deficiency was described [88]. As an infant this patient had vaccine-associated disseminated BCG infection which was cured with antibiotics. Over one decade later she developed disseminated *M. abscessus* infection which could not be controlled with antibiotics but was cured after the addition of adjunctive subcutaneous IFNγ. Both infections were associated with mature paucibacillary granulomas. Genetic analysis showed a homozygous nucleotide substitution in IFNGR2 causing an amino acid substitution in the extracellular protein domain (R114C). IFNγR2 protein was present on the surface of patient monocytes, and IFNγ responsiveness was diminished but not abolished. This case report established that the genotype-phenotype correlations established for IFNGR1 also apply to IFNGR2. This case also raises intriguing questions about the nature of the interactions between IFNγR1 and IFNγR2 in the IFNγ receptor complex.

## 6. IL12 and IL12 receptor deficiency

Patients with severe mycobacterial disease and autosomal recessive mutations in the genes encoding IL-12 p40 [90] or IL-12Rβ1 [91,92] have recently been identified (Table 1). In each case, the mutation precluded protein expression. Each patient suffered from severe infection with either NTM or vaccine-associated BCG, and most had severe *Salmonella* infections. However, in most instances, infection was effectively treated with antibiotics. In several patients, administration of adjunctive IFNγ along with antibiotics was associated with substantial clinical improvement. Well-organized, mature, tuberculoid granulomas were observed on histopathologic examination of affected tissues from IL-12Rβ1-deficient patients [91,92], suggesting that IL-12-dependent IFNγ induction is not required for mature granuloma formation. Tuberculin-specific DTH testing was normal in IL-12Rβ1-deficient patients with BCG infection [92], implying that, like IFNγ, IL-12 is not required for development of DTH. In vitro, activated T lymphocytes and NK cells from patients had markedly diminished but not absent IFNγ production [90–92]. This residual IL-12-independent IFNγ production in IL-12p40 and IL-12Rβ1 deficient patients may account for their milder clinical phenotype compared with patients with complete IFNγ receptor deficiency. Findings in IL-12p40-, and IL-12Rβ1-deficient patients further support that IFNγ is critical in control of mycobacteria and *Salmonella* infections, and that a principal role of IL-12 in control of these infections is to stimulate IFNγ production.

## 7. Human IFNγ deficiency?

To date human IFNγ deficiency has not been described, despite identification of at least ten different human IFNγ receptor mutations. The current model of IFNγ ligand-receptor interactions does not provide a ready explanation for this discrepancy. The IFNγ knockout mouse model indicates that in mice, IFNγ is not required for normal growth and development. Moreover, disease due to experimental infection with HSV1 or vaccinia virus is less severe in IFNγ knock-

out mice than in IFNγR1 knockout mice [64]. Immunologic and genetic evaluation of more patients with heightened susceptibility to infections caused by intracellular pathogens may shed light on this issue. If human IFNγ deficiency does exist and is associated with a clinical phenotype, then it may be correctable with administration of exogenous IFNγ.

## 8. Tuberculosis in IFNγ receptor, IL-12Rβ1, and IL-12 p40 deficient patients

Among the described patients with known IFNγ or IL-12 pathway defects, only one case of probable tuberculosis has been diagnosed [87]. In a 3-year-old girl with AR partial IFNγR1 deficiency who developed cough, pneumonia, and erythema nodosum, a clinical diagnosis of tuberculosis was made on the basis of development of delayed-type hypersensitivity to tuberculin purified protein derivative and clinical response to administration of anti-tuberculosis antibiotics. Unfortunately, no microbiologic diagnosis was made. A possible second case is the mother of two children with autosomal dominant partial IFNGR1 818del4 mutations. She reportedly died at age 33 of disseminated tuberculosis after three episodes of invasive tuberculosis [89]. However, genetic material was not available and her genotype therefore remains unknown.

The role of IFNγ and IL-12 pathway defects in human susceptibility to tuberculosis is clearly an important issue, given that tuberculosis remains a leading cause of infectious disease mortality worldwide. The apparent low incidence of tuberculosis in patients with IFNγ or IL-12 pathway defects may be due to a combination of lack of exposure in patients described to date (most of whom live in developed countries where the incidence of tuberculosis is low), and lack of genetic evaluation in patients with tuberculosis who live in developing countries where the incidence of tuberculosis is higher. Alternatively, human host defense against *M. tuberculosis* may not be dependent on IFNγ or IL-12 pathways, although this seems unlikely. As more patients with IFNγ or IL-12 pathway defects are identified, this issue may be resolved. It will be important to determine if subtle functional changes due to gene polymorphisms are sufficient to confer protection against poorly pathogenic mycobacteria but insufficient for protection against virulent organisms like *M. tuberculosis*.

## 9. Nonmycobacterial infections in IFNγ receptor, IL-12Rβ1, and IL-12 p40 deficient patients

While mycobacterial infections have been the major recognized cause of morbidity and mortality in IFNγ receptor, IL-12Rβ1, and IL-12 p40 deficient patients, infections with other intracellular microorganisms have been described. Severe infections with *Salmonella* species have been diagnosed in a small number of reported IFNγ receptor deficient patients [78,87], 70% of reported IL-12Rβ1 deficient patients [91,92], and the single reported IL-12 p40 deficient patient [90]. One patient with *L. monocytogenes* meningitis [85], one patient with refractory disseminated *Histoplasma capsulatum* infection [89], and two siblings with pneumonitis thought due to *Mycoplasma pneumoniae* (one of whom also had serologic evidence for a pneumonia due to a *Legionella* species) [87] have also been reported. The severity of some viral infections (including herpes viruses, parainfluenza, and respiratory syncytial virus) is increased in some patients with IFNγ receptor deficiency [95]. The increased severity of herpes virus infections parallels the heightened susceptibility of IFNγ and IFNγ receptor knockout mice to herpes viruses [55,56]. However, some patients with IFNγ receptor deficiency have had normal recovery from infections caused by RSV and varicella, or immune serologies for HSV, EBV, and CMV without histories of clinical disease [62]. These observations support a role for IFNγ in human host defense against some viral infections, but indicate that for viral infections, but not infections due to poorly pathogenic mycobacteria, other immunologic mechanisms may compensate in the absence of IFNγ responsiveness. As more children with IFNγ pathway defects are identified, a broader spectrum of infection susceptibility may become apparent.

## 10. Conclusions

Identification of humans with mutations in genes for IFNγ receptor proteins, IL-12 p40, and IL-12Rβ1 has highlighted the importance of IFNγ pathways in human host defense against intracellular pathogens including mycobacteria, *Salmonella*, and some viruses. Phenotype to genotype correlations are emerging as more patients are identified. In patients with IFNγ receptor deficiency, phenotype, as assessed by infection severity and histopathology, is related to degree of IFNγ responsiveness. Children with complete absence of IFNγ responsiveness typically have severe disseminated mycobacterial infections, with lepromatoid granulomas in affected tissues. Patients with partial IFNγ responsiveness due to either AR or AD IFNγ receptor mutations usually have less severe mycobacterial disease associated with tuberculoid granulomas. IL-12 p40 deficiency and IL-12Rβ1 deficiency are also associated with heightened susceptibility to infections with BCG, NTM, and *Salmonella*, although the clinical

phenotype is typically milder than that of complete IFNγ receptor deficiency.

## 11. Future directions

Recognition of IFNγ's role in human host defense against intracellular pathogens emphasizes the importance of research to understand the mechanisms by which IFNγ activates macrophage killing of intracellular organisms, and the mechanisms by which pathogens such as *M. tuberculosis* apparently circumvent macrophage killing. Better understanding these mechanisms will lead to the development of rational preventive and therapeutic strategies directed against *M. tuberculosis* and other intracellular pathogens. It is intriguing to speculate that genetic changes causing subtle functional disturbances in IFNγ or IL-12 pathways might contribute to tuberculosis susceptibility at the population level.

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