

# Mendelian susceptibility to mycobacterial infection in man

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## Summary

Selective susceptibility to weakly pathogenic mycobacteria, such as bacillus Calmette-Guérin (BCG) vaccine and environmental non-tuberculous mycobacteria (NTM), has long been suspected to be a Mendelian disorder but its molecular basis remained elusive. Recently, mutations in the interferon- $\gamma$  receptor ligand-binding chain (IFN $\gamma$ R1), interferon- $\gamma$  receptor signaling chain (IFN $\gamma$ R2), Signal Transducer and Activator of Transcription-1 (STAT-1), interleukin-12 p40 subunit (IL-12 p40), and interleukin-12 receptor  $\beta$ 1 chain (IL-12R $\beta$ 1) genes have been identified in a number of patients with severe BCG or NTM infection. Dominant or recessive alleles causing

complete or partial cellular defects have been found to define nine different inheritable disorders. Although genetically distinct, these conditions are immunologically related and highlight the essential role of interferon  $\gamma$ -mediated immunity in the control of mycobacteria in man. The genetic and immunologic heterogeneity of this syndrome makes accurate diagnosis challenging but vital as decisions about the most appropriate treatment are best taken based on an accurate molecular diagnosis.

*Key words: mycobacterial infection; BCG; non-tuberculous mycobacteria*

## Introduction

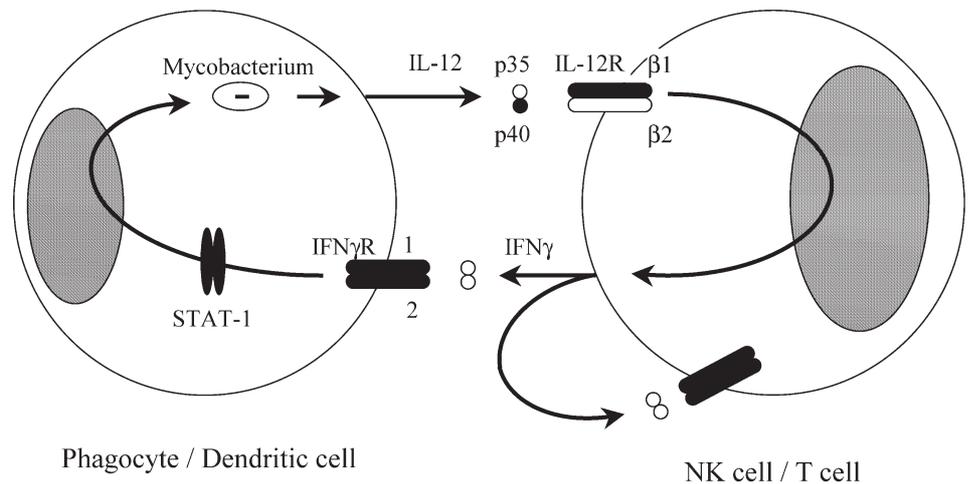
Bacillus Calmette-Guérin (BCG) vaccines and environmental non-tuberculous mycobacteria (NTM) are leading causes of severe disease in immunocompromised children. They may also cause severe disease in otherwise healthy children with no overt immunodeficiency [1-4]. Patients with mycobacterial disease associated with immunodeficiency [5] are susceptible to a wide variety of viruses, bacteria, fungi, and protozoans. In contrast, patients with so-called idiopathic BCG and NTM infections do not generally have associated infections, apart from salmonellosis which affects less than half of the cases. Parental consanguinity and familial forms are frequently observed, therefore, this syndrome was designated as «Mendelian susceptibility to mycobacterial infection» (Mendelian Inheritance in Man Number 209950) [6]. The syndrome does not seem to be confined to a particular ethnic group or geographic region, and over one hundred patients have already been reported. The prevalence of idiopathic disseminated BCG infection in France has been estimated to be at least 0.59 cases per million children vaccinated [2].

Although the clinical features seem to be restricted to a predisposition to mycobacterial infection, the syndrome appears to be heterogeneous. Firstly, the genetic basis of the syndrome is not the same in all affected families. In most familial cases, inheritance is autosomal and recessive, but X-linked recessive inheritance seems to be involved in one family [3, 7] and autosomal dominant inheritance has been reported for three other families [8, 9]. Secondly, clinical outcome differs between children and has been found to correlate with the type of BCG granulomatous lesion present [10]. Children with lepromatous-like granulomas (poorly delimited, multibacillary, with no epithelioid or giant cells) generally die of overwhelming infection, whereas children with tuberculoid granulomas (well delimited, paucibacillary, with epithelioid and giant cells) have a favorable outcome.

Five genes have been found to be mutated in children with this syndrome: *IFNGR1* and *IFNGR2*, encoding the two chains of the receptor for IFN $\gamma$ , a pleiotropic cytokine secreted by NK and T cells; *STAT1*, encoding an essential mole-

**Figure 1**

Genetic etiologies of Mendelian susceptibility to mycobacterial infection. Cells involved in IFN $\gamma$ -mediated immunity. IL-12 is principally secreted by phagocytes and dendritic cells and binds to a heterodimeric receptor consisting of  $\beta 1$  and  $\beta 2$  chains, which is specifically expressed on NK and T lymphocytes. IFN $\gamma$  is secreted by NK and T lymphocytes and binds to a ubiquitous receptor made of two chains, a ligand-binding (IFN $\gamma$ R1) and a signaling-associated chain (IFN $\gamma$ R2). STAT-1 is phosphorylated in response to IFN $\gamma$  and is translocated to the nucleus as a homodimer. Five molecules (in black) have been found to be mutated in certain patients with severe mycobacterial infection.



cule in the IFN $\gamma$ R signaling pathway; *IL12P40*, encoding the p40 subunit of IL-12, a potent IFN $\gamma$ -inducing cytokine secreted by macrophages and dendritic cells; and *IL12RB1*, encoding the  $\beta 1$  chain of the receptor for IL-12, expressed on NK and T cells (figure 1). The type of mutation may also partly account for clinical heterogeneity: dominant and recessive mutations have been found in one gene (*IFNGR1*), and null mutations and mutations with mild effects have been found in two genes (*IFNGR1* and *IFNGR2*). Complete IFN $\gamma$ R1 deficiency is associated with two types of null mutations. These mutations define nine disorders, which have a common pathogenic mechanism, i.e. the impairment of IFN $\gamma$ -mediated immunity. Impaired secretion of IFN $\gamma$  occurs in IL-12p40 and IL-12R $\beta 1$  deficiencies, and impaired response to IFN $\gamma$  occurs in IFN $\gamma$ R1, IFN $\gamma$ R2 and STAT-1 deficiencies.

IFN $\gamma$  being a major macrophage-activating cytokine, it is likely that macrophages play a key role in the pathogenesis of mycobacterial infections in the patients [11]. However, lymphocytes may also be involved, either directly, as they express IFN $\gamma$ R molecules, or indirectly, because impaired macrophage activation by IFN $\gamma$  may restrict lymphocyte activation by other cytokines. The level of secretion of both monokines (TNF $\alpha$ , IL-

12) and lymphokines (IFN $\gamma$ ) by peripheral blood mononuclear cells has been reported to be low in IFN $\gamma$ R1-deficient children [12, 13]. Typically, these patients do not develop well differentiated and well circumscribed mycobacterial granulomas [10, 14]. This provides further evidence that impaired IFN $\gamma$ -mediated immunity affects both phagocytes and lymphocytes.

The onset of symptoms generally occurs in childhood (although the disease may progress in adulthood), and therefore patients are most frequently seen by paediatricians. The rarity and heterogeneity of the syndrome are a major challenge for accurate diagnosis and treatment. In addition, the clinical boundaries of the syndrome and the clinical features associated with the eight known underlying genetic defects are ill-defined. Finally, certain patients with the syndrome have no defect in any of the four genes identified to date. We shall therefore briefly review the nine known inheritable disorders underlying severe mycobacterial infection, and attempt to provide guidelines for their diagnosis and management, based on our experience and published reports and reviews [15, 16]. More basic aspects (genetics, immunology, microbiology, animal models) of these conditions have been reviewed elsewhere [11, 17, 18].

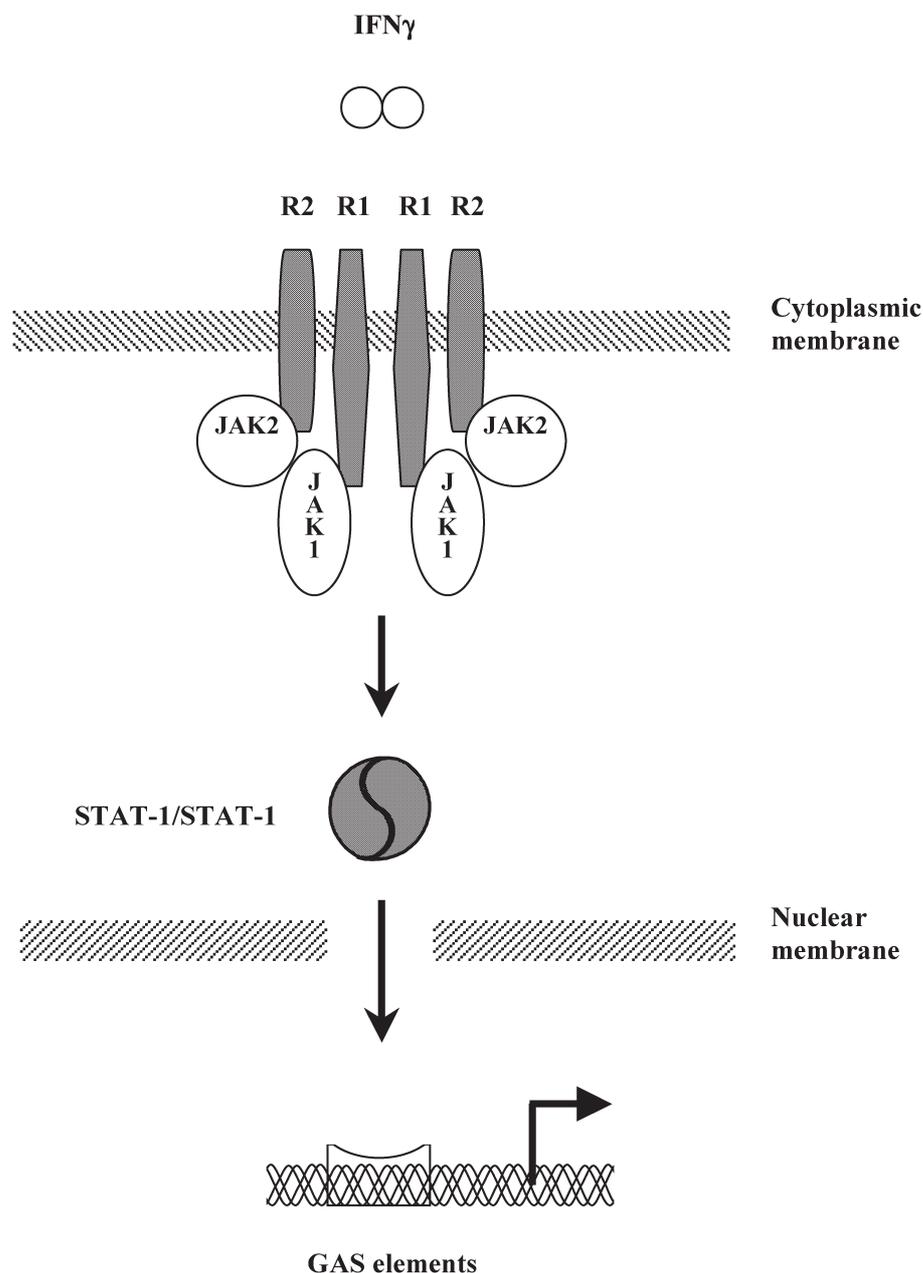
## Two forms of complete recessive IFN $\gamma$ R1 deficiency

Complete interferon- $\gamma$  receptor ligand-binding chain (IFN $\gamma$ R1) deficiency was the first genetic aetiology to be identified in two kindreds [12, 14] (figure 2). Six further families have since been reported [13, 15, 19–22], raising the number of reported affected kindreds to eight and of patients to fifteen. The *IFNGR1* mutations identified are recessive, and both homozygous and compound heterozygous patients have been found. The families originated from various countries and no recurrent mutations have been observed. The mutations are

null as they preclude cell surface expression of the receptor. A lack of cellular responses to IFN $\gamma$  *in vitro* has been demonstrated in these patients, and the mutant alleles have been shown to cause the impaired cellular response to IFN $\gamma$  in experiments involving the complementation of defective cells with the wild-type *IFNGR1* gene. Four other unrelated families have recently been identified in which the children (a total of five) with complete IFN $\gamma$ R1 deficiency were found to have expression of IFN $\gamma$ R1 molecules on the cell surface [23, 24].

**Figure 2**

Genetic defects in the IFN $\gamma$ -signaling pathway. IFN $\gamma$  is a homodimer that binds to and dimerises IFN $\gamma$ R1 molecules. Upon dimerisation, IFN $\gamma$ R2 molecules are recruited to form a tetramer which brings together IFN $\gamma$ R1- and IFN $\gamma$ R2-associated JAK-1 and JAK-2 kinases, respectively. Phosphorylation of IFN $\gamma$ R1 allows the docking of STAT-1, a latent cytosolic monomer. The two STAT-1 molecules recruited are subsequently phosphorylated and released into the cytosol, where they dimerise and are translocated to the nucleus. Three molecules (in black) in the signaling pathway have been found to be mutated in patients with severe mycobacterial infection.



Mutations in *IFNGR1* were identified in these patients, and prevented the binding of the encoded surface receptors to their natural ligand, IFN $\gamma$ .

Infections due to BCG and/or NTM were diagnosed in all patients with IFN $\gamma$ R1 deficiency. In particular, infection with *M. smegmatis* has been reported. This species is one of the least virulent mycobacteria and had not been previously reported to cause disseminated disease in humans. In all cases, mycobacterial infections occurred before the age of three years, causing death in nine patients and requiring continuous antimycobacterial treatment in the eight survivors. Salmonella infection was reported in two children only, one of whom had undergone a splenectomy. Severe disease due to *Listeria monocytogenes* was reported in one patient, and severe cytomegalovirus infection in two others. However, other factors, such as disseminated mycobacterial infection itself, may have been involved in predisposition to viral disease. No other opportunistic infections were observed, and the course

of infections due to common childhood pathogens was unremarkable. Complete IFN $\gamma$ R1 deficiency therefore seems to result in a selective susceptibility to the early-onset of a severe mycobacterial infection. Nevertheless, the clinical phenotype may be expanding, as suggested by the recently observed viral infections [25]. A comprehensive, multi-institutional clinical and microbiological survey is therefore needed.

A diagnosis of complete IFN $\gamma$ R1 deficiency should be considered for children with severe mycobacterial disease (BCG, NTM) occurring before the age of three to five years. No asymptomatic patients over 3 years of age have been reported. Lepromatous-like lesions, particularly in response to BCG, are suggestive of the absence of IFN $\gamma$ -mediated immunity, whereas tuberculoid granulomas almost certainly rule out complete IFN $\gamma$ R1 deficiency. A lack of detectable IFN $\gamma$ R1 molecules on freshly prepared peripheral blood mononuclear cells or cultured EBV-transformed B cells strongly

suggests complete IFN $\gamma$ R1 deficiency. Conversely, the detection of surface IFN $\gamma$ R1 molecules by flow cytometry with specific monoclonal antibodies does not exclude the diagnosis of complete IFN $\gamma$ R1 deficiency, since some mutations do not impair surface expression and recognition by commercial antibodies but do prevent IFN $\gamma$  binding and subsequent signaling. Functional studies aimed at determining the cellular responses to recombinant IFN $\gamma$  can be used as complementary methods for diagnosis. For all functional assays it is important to use high concentrations of IFN $\gamma$  in order to differentiate patients with partial IFN $\gamma$ R1 deficiency (who respond to high concentrations). Biochemical and functional studies may suggest complete IFN $\gamma$ R1 deficiency, but accurate diagnosis requires conclusive genetic studies.

The molecular diagnosis of complete IFN $\gamma$ R1 deficiency has major therapeutic implications. Efforts should be made to identify the pathogenic

mycobacterial species and determine its susceptibility to antibiotics. Mycobacterial infections should be treated aggressively with at least four different drugs. Antibiotic treatment should not be discontinued. Occasionally, surgery to remove a refractory infectious site, such as the spleen or abdominal lymph nodes, may be effective. Bone marrow transplantation was thought to be the treatment of choice because IFN $\gamma$  treatment is ineffective, due to the lack of specific receptors and since mycobacterial infections are overwhelming. Patients with IFN $\gamma$ R1 deficiency should undergo transplantation only if clinical remission of mycobacterial infection is obtained (complete microbiological remission is probably never achieved). However, it appears that bone marrow transplantation in children with complete IFN $\gamma$ R deficiency is associated with considerable morbidity and mortality (Roesler J, personal communication).

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## Complete IFN $\gamma$ R2 deficiency

One child with complete IFN $\gamma$  receptor signaling chain (IFN $\gamma$ R2) deficiency has been first reported [26]. A homozygous recessive frameshift deletion was found in the *IFNGR2* coding region, resulting in a premature stop codon upstream from the segment encoding the transmembrane domain. No mutation was found in the *IFNGR1* gene and cell surface expression of IFN $\gamma$ R1 was normal. No lack of cell surface IFN $\gamma$ R2 expression was documented (the staining of control cells with available specific antibodies was poor), but a lack of cellular response to IFN $\gamma$ , as detected by STAT-1 phosphorylation, was demonstrated. The child had early-onset and severe infections due to *M. avium* and *M. fortuitum*, requiring continuous multi-drug antimycobacterial therapy. No mature granulomas were observed. An episode of curable Herpes Simplex Virus esophagitis was also reported. Another child with complete IFN $\gamma$ R2 deficiency has recently been identified by the same authors [15]. This disorder demonstrates that human IFN $\gamma$ R2, like IFN $\gamma$ R1, is required *in vivo* for IFN $\gamma$ -mediated signaling and antimycobacterial protective immunity.

Thus, null recessive *IFNGR2* mutations, like

null recessive *IFNGR1* mutations, may be responsible for early-onset and severe mycobacterial infection with impaired granuloma formation. More patients must be studied to describe accurately the histological lesions, clinical features, and outcome of children affected by this condition. However, the available data suggest that complete IFN $\gamma$ R2 deficiency closely resembles complete IFN $\gamma$ R1 deficiency. The criteria for suspicion of complete IFN $\gamma$ R1 deficiency therefore also apply to complete IFN $\gamma$ R2 deficiency. The exclusion of IFN $\gamma$ R1 deficiency by sequencing the *IFNGR1* gene coding region in a patient whose cells do not respond to high concentrations of IFN $\gamma$  *in vitro* strongly suggests a diagnosis of complete IFN $\gamma$ R2 deficiency, although further tests are required to confirm the diagnosis. Complete IFN $\gamma$ R2 deficiency cannot be reliably diagnosed using commercially available IFN $\gamma$ R2-specific antibodies, because control cells are only weakly stained with these antibodies. The *IFNGR2* exons and flanking intron regions must therefore be sequenced for reliable diagnosis. The recommended treatment for patients with complete IFN $\gamma$ R2 deficiency is the same as that for patients with complete IFN $\gamma$ R1 deficiency.

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## Partial recessive IFN $\gamma$ R1 deficiency

Two siblings with partial, rather than complete, IFN $\gamma$ R1 deficiency have also been reported [27]. A homozygous recessive missense mutation causing an amino-acid substitution in the extracellular domain of the receptor was identified. The encoded receptor was detected with specific antibodies and found to be normally expressed at the surface of monocytes. Cells from healthy children

respond to IFN $\gamma$  at low concentrations and cells from children with complete IFN $\gamma$ R1 deficiency do not respond to IFN $\gamma$ , even at high concentrations. In contrast, blood cells and EBV-transformed B cells from the children with partial IFN $\gamma$ R1 deficiency respond to IFN $\gamma$ , but only at high concentrations. Together, these experiments suggest that the missense *IFNGR1* mutation re-

duces the affinity of the encoded receptor for its ligand, IFN $\gamma$ . The pathogenic role of the mutation has been demonstrated by gene transfer experiments. Recipient cells from a child with complete IFN $\gamma$ R1 deficiency transfected with the *IFNGR1* missense mutant gene, responded to high, but not to low, concentrations of IFN $\gamma$ .

One child had disseminated BCG and *Salmonella enteritidis* infections with a favorable outcome. The sibling, who had not been vaccinated with BCG, had curable symptomatic primary tuberculosis. Both are currently well at 16 and 19 years of age, with no treatment. The occurrence of clinical tuberculosis in one child who had not been vaccinated with BCG suggests that IFN $\gamma$ R1-deficient children are also susceptible to tuberculous mycobacteria. The clinical phenotype of the two siblings with partial IFN $\gamma$ R1 deficiency appears to be milder than that of children with complete IFN $\gamma$ R1 deficiency. Unlike children with complete IFN $\gamma$ R1 deficiency, these children had well-circumscribed and well-differentiated tuberculoid BCG granulomas. This suggests that IFN $\gamma$ R1-mediated signaling is able to promote morphologically mature granuloma formation if it is impaired but not completely abolished. The occurrence of mycobacterial infections, however, suggests that the granulomas form later than normal, are poorly functional, or are insufficient in number. Thus, there is a correlation between the genotype (null or mild mutation), the cellular phenotype (complete or partial defect), the histological phenotype (immature or mature granulomas), and the clinical phenotype (poor or favorable outcome).

A diagnosis of partial recessive IFN $\gamma$ R1 deficiency should be considered in young children with BCG infection and mature granulomas (mature granulomas are not observed in children with complete IFN $\gamma$ R deficiency). It should also be considered in unusually severe cases of tuberculosis (particularly, but not exclusively, in patients who were not vaccinated with BCG). Patients with partial IFN $\gamma$ R1 deficiency are probably also prone to NTM infection. Patients with complete or partial IFN $\gamma$ R1 deficiency may respond to antimycobacterial treatment, but neither the extent of dissemination nor the response to treatment are sufficient to determine the diagnosis. Diagnosis cannot be based purely on the detection of IFN $\gamma$ R1 at the cell surface, because normal numbers of molecules are detected on blood cells. Functional assays are suggestive, showing a response to high but not to low IFN $\gamma$  concentrations. Reliable diagnosis requires sequencing of the gene. However, depending on the type of *IFNGR1* mutation identified, gene transfer experiments may be required to confirm the involvement of mutations. Patients should be followed up and mycobacterial infections should be promptly treated with antibiotics, which may later be discontinued. As the two siblings with partial IFN $\gamma$ R1 deficiency are thriving in the absence of treatment, prophylactic IFN $\gamma$  therapy and antibiotics, as well as bone marrow transplantation are not indicated. As IFN $\gamma$  induces signaling events *in vitro*, IFN $\gamma$  therapy is probably the best option for patients with partial recessive IFN $\gamma$ R1 deficiency who may suffer from mycobacterial disease refractory to antibiotics.

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## Partial recessive IFN $\gamma$ R2 deficiency

A 20-year-old patient with a history of BCG and *M. abscessus* infections was found to have partial, as opposed to complete, IFN $\gamma$ R2 deficiency [28]. A homozygous nucleotide substitution was found in *IFNGR2*, which caused a single amino-acid substitution in the extracellular region of the encoded receptor. Membrane-bound IFN $\gamma$ R2 were weakly but significantly detected on EBV-transformed B cells from the patient and a control by flow cytometry with a specific antibody, whereas no staining was detected on B cells from a patient with complete IFN $\gamma$ R2 deficiency. Nuclear translocation of STAT-1 in the patient's EBV-transformed B cells and cell surface expression of HLA-DR in SV40-transformed fibroblasts were found to be impaired following stimulation with IFN $\gamma$ . Neither was, however, completely abolished. Transfection with the wild-type *IFNGR2* gene restored full responsiveness to IFN $\gamma$ . Thus, there is a causal relationship between the *IFNGR2* missense mutation and weak cellular responses to IFN $\gamma$ . The molecular mechanism remains to be determined. This case illustrates, as for IFN $\gamma$ R1 deficiency, that there is a strict correlation between

the *IFNGR2* genotype and the cellular, histological, and clinical phenotype. The level of IFN $\gamma$ -mediated immunity seems to be the crucial factor determining the histopathological lesions associated with, and the clinical outcome of, mycobacterial infections.

A diagnosis of partial IFN $\gamma$ R2 deficiency should be considered in children with a mild histological and clinical phenotype. Impaired, but not abolished, cellular responses to IFN $\gamma$  *in vitro* are further suggestive of the condition. Reliable diagnosis can be made only by sequencing the *IFNGR2* gene. Studies of the expression of the IFN $\gamma$ R2 chain with commercially available specific antibodies are not reliable for diagnosis. Gene transfer experiments may also be required, depending on the mutation identified. Patients should be followed up and mycobacterial infections should be promptly treated with antibiotics and, if needed, IFN $\gamma$ . Antibiotics may be discontinued after several months of complete clinical remission. Curative IFN $\gamma$  treatment has been successful in the sole patient identified to date, but a long-term prophylactic regimen based on IFN $\gamma$  is probably not nec-

essary. Bone marrow transplantation is clearly not indicated, given the apparently good prognosis of this condition. Treatment should however be dis-

cussed on a case by case basis, according to the patient's cellular, histological, and clinical phenotype.

## Partial dominant IFN $\gamma$ R1 deficiency

Eighteen patients from twelve unrelated kindreds were found to have a dominant form of partial IFN $\gamma$ R1 deficiency [8]. These patients have a heterozygous frameshift small deletion in *IFNGR1* exon 6, downstream from the segment encoding the transmembrane domain. The mutant alleles encode truncated receptors with no more than five intracellular amino-acids. The receptors reach the cell surface because the leader extracellular and transmembrane domains are conserved. They bind IFN $\gamma$  with normal affinity because the extracellular region is properly folded. The receptors dimerize and form a tetramer with two IFN $\gamma$ R2 molecules, but they do not transduce IFN $\gamma$ -triggered signals due to the lack of intracellular binding sites for the cytosolic molecules (JAK-1 and STAT-1) involved in the signaling cascade. The receptors also accumulate at the cell surface due to the lack of an intracellular recycling site. The combination of normal binding to IFN $\gamma$ , abolished signaling in response to IFN $\gamma$ , and accumulation of receptors at the cell surface accounts for their dominant-negative effect. Most IFN $\gamma$ R1 dimers in heterozygous cells are non-functional due to the presence of at least one defective molecule. The few wild-type IFN $\gamma$ R1 dimers that form in response to IFN $\gamma$  account for the defect being partial rather than complete. Indeed, IFN $\gamma$  triggers residual cellular responses. An interesting genetic feature of this disorder is that position 818 of *IFNGR1* is the first small deletion hotspot to be identified in the human genome. Overlapping small deletions (818del4 in eleven kindreds and 818delT in one) were found to occur independently in twelve unrelated families. Two other dominant *IFNGR1* mutations were recently reported, resulting in a similar phenotype [15, 29].

The severity of the clinical features of patients with partial dominant IFN $\gamma$ R1 deficiency appear to be intermediate between those of the complete and partial recessive deficiencies. Patients are generally vulnerable to BCG, but two children were vaccinated with no adverse effect. NTM infections are frequent, but generally after the age of three years. Complete remission can be achieved with antimycobacterial drugs, although IFN $\gamma$  is also required in some cases. Patients may experience recurrent infections with the same mycobacterial

species, or with different mycobacterial species. Only three of the 18 patients died, and the survivors are currently aged between 4 and 60 years. BCG granulomas are invariably mature, whereas NTM granulomas may be immature. Interestingly, mycobacterial lesions of the bones seem to be particularly frequent (15 of 18 patients), whereas they are rarely observed in patients with other types of IFN $\gamma$ R deficiency. One patient also had severe disease due to *Histoplasma capsulatum*, and another patient with a related but different dominant *IFNGR1* mutation was found to have severe disease caused by Varicella Zoster Virus.

A diagnosis of dominant partial IFN $\gamma$ R1 deficiency should be considered in children with BCG infection and mature granulomas, and in patients with NTM infection. Patients who respond to IFN $\gamma$  therapy are also good candidates, as patients with complete IFN $\gamma$ R deficiency do not respond. However caution is advisable in interpretation because coincidental improvement may occur in patients with complete IFN $\gamma$ R deficiency. NTM infection generally occurs in patients over 3 years of age (earlier in patients with complete deficiency). Autosomal dominant inheritance of the clinical syndrome in the family studied clearly suggests the diagnosis. The detection of a much larger than usual number of receptors at the cell surface greatly facilitates the diagnosis of dominant partial IFN $\gamma$ R1 deficiency. Five to ten times the normal number of IFN $\gamma$ R1 molecules are readily detected on various cell types (freshly prepared monocytes and lymphocytes, cultured EBV-transformed B cells). Mutations causing partial dominant IFN $\gamma$ R1 deficiency are known to occur in the vicinity of nucleotide position 818. Thus, the amplification and sequencing of exon 6 around position 818 is a simple way to confirm the diagnosis. Treatment options include antibiotics and IFN $\gamma$ , both of which are effective in patients with dominant partial IFN $\gamma$ R1 deficiency. Efforts should be made to identify the pathogenic mycobacterial species and to determine its susceptibility to antimycobacterial drugs, in order to determine the most appropriate treatment. It is unclear whether prophylactic treatment is required. Given the good prognosis, bone marrow transplantation is not indicated.

## Partial STAT-1 deficiency

Two kindreds with the same heterozygous mutation in *STAT1* causing partial dominant STAT-1 deficiency have recently been described [9]. STAT-1 is a critical transducer of IFN-mediated signals, either as STAT-1 homodimers, designated gamma-activating factor (GAF) or as STAT-1/STAT-2/p48 trimers, known as interferon-stimulated gamma factor 3 (ISGF3). This *STAT1* mutation decreased cellular responses to both IFN $\gamma$  and IFN $\alpha$  in terms of the activation of GAF, but not ISGF3. This mutation results in a loss of function for both cellular phenotypes, but is dominant for GAF and recessive for ISGF3 activation in the patients' heterozygous cells stimulated with IFNs. Clinically, one patient suffered from disseminated BCG infection with tuberculous granulomas, whereas the other had disseminated *M. avium* infection. They are now 10 and 36 years old and well.

The lack of viral illness suggests that IFN-mediated viral immunity is STAT-1-independent and/or ISGF3-dependent. The clinical and cellular phenotypes of the patients were similar to those of patients with partial recessive IFN $\gamma$ R deficiency, in terms of mycobacterial disease and GAF activation, respectively. This further documents the strict genotype-phenotype correlation in the IFN $\gamma$  signaling pathway, with complete lack of response to IFN $\gamma$  *in vitro* associated with a severe clinical outcome *in vivo*, and partial lack of response to IFN $\gamma$  with a good clinical outcome [30]. Moreover, this observation implies that human IFN $\gamma$ -mediated mycobacterial immunity is dependent on STAT-1 and GAF. Clinically, these patients should be followed up and mycobacterial infections must be treated with antibiotics and IFN $\gamma$ . Bone marrow transplantation is not indicated.

## Complete IL-12 p40 deficiency

A child with a recessive mutation in the gene encoding the p40 subunit of IL-12, a potent IFN $\gamma$ -inducing heterodimeric cytokine (p70) secreted by macrophages and dendritic cells, has been reported [31]. The mutation consists of a homozygous frameshift deletion of 4.4 kb encompassing two coding exons. Neither p40 nor p70 was detected in the supernatants of BCG-activated phagocytes and CD40-ligand-activated dendritic cells from the patient. Transfection of a defective cell line with the wild-type *IL12P40* gene led to the secretion of IL-12 p40 and p70. This implies that there is a causative relationship between the *IL12P40* homozygous deletion and the lack of IL-12 production. Another kindred with impaired, but not abolished, IL-12 production has also been reported [6, 7]. The genetic defect was not identified, but the familial pedigree does not seem to be consistent with autosomal recessive IL-12 p40 deficiency, suggesting that another genetic defect may be responsible for the IL-12 deficiency.

The patient with complete IL-12 deficiency had curable BCG and *Salmonella enteritidis* infections. His lymphocytes secreted lower than normal amounts of IFN $\gamma$  following the stimulation of peripheral blood mononuclear cells *in vitro* by PHA or BCG. Impaired IFN $\gamma$  secretion was complemented in a dose-dependent manner by exogenous recombinant IL-12, implying that IFN $\gamma$  deficiency is not a primary event but a consequence of inherited IL-12 deficiency. IFN $\gamma$  therapy has been ef-

fective for treating and preventing mycobacterial infections in IL-12 deficient children. Thus, several lines of evidence strongly suggest that IL-12-deficient children suffer from mycobacterial infection primarily because their IFN $\gamma$ -mediated immunity is impaired. Residual IL-12-independent secretion of IFN $\gamma$  probably accounts for the clinical phenotype being milder than that of children with complete IFN $\gamma$ R deficiency.

A diagnosis of IL-12 deficiency should be considered for children with the mild form of the syndrome. If the expression and function of the two IFN $\gamma$ R chains are normal, IFN $\gamma$  production by PHA- or BCG-stimulated peripheral blood mononuclear cells should be quantified *in vitro* using commercially available specific antibodies in a simple ELISA. IL-12-deficient patients produce one tenth to one hundredth the normal amount of IFN $\gamma$ . A lack of IL-12 p70 and p40 production by phagocytes stimulated with BCG can be detected in an ELISA. Gene sequencing should ultimately provide definitive diagnostic data. Children with IL-12 deficiency may respond to IL-12 treatment, but such treatment was not tested in the patients with IL-12 deficiency identified to date. IFN $\gamma$  treatment has been remarkably effective, and there is no indication for bone marrow transplantation. As for patients with partial IFN $\gamma$ R deficiency, prophylactic therapy with antibiotics or IFN $\gamma$  is probably not necessary, but this should be determined on a case by case basis.

## Complete IL-12R $\beta$ 1 deficiency

Mutations in the gene encoding the  $\beta$ 1 subunit of the IL-12 receptor have been identified in eleven children [32–36]. All patients were homozygous for recessive mutations (nonsense, missense and splice mutations). The families were from different countries and the mutations differed from each other. These mutations preclude the cell surface expression of IL-12R $\beta$ 1 on activated T cells, with no such receptors detected by flow cytometry with specific antibodies. A lack of expression on NK cells has been predicted but not yet investigated. Nevertheless, neither NK cells nor T cells were found to be responsive to IL-12 stimulation *in vitro*. Molecular complementation of defective cells by transfection with wild-type *IL12RB1* gene has been reported in two kindreds [35–36]. Missense mutations have thus been experimentally validated in these two kindreds only.

The clinical phenotype appears to be less severe than that of children with complete IFN $\gamma$ R deficiency, as BCG infections were curable in the three patients with BCGosis and the NTM infections (diagnosed in seven patients) occurred after the age of three years in four patients (including three cases diagnosed in adulthood). Remarkably, one patient who was resistant to BCG despite three inoculations of live vaccine and did not develop atypical mycobacteriosis, had abdominal tuberculosis at 18 years of age [36]. One of the patients died of NTM infection. The other eight patients were well at the last follow up. Five of the seven patients had associated salmonella infections, but despite exposure to many infectious agents no other infections were reported. The histological phenotype also appears to be milder, as BCG granulomas were found to be well-delimited and well-differentiated. NTM granulomas were generally less mature and multibacillary.

IFN $\gamma$  secretion *in vitro* by otherwise functional NK cells and T cells has been demonstrated to be impaired in patients with complete IL-12R $\beta$ 1 deficiency. IFN $\gamma$  treatment has been found to be effective for controlling mycobacterial infection. Thus, as in IL-12-deficient children, impaired IFN $\gamma$  secretion is probably responsible for mycobacterial disease in IL-12R $\beta$ 1-deficient children and residual, IL-12-independent, IFN $\gamma$ -mediated immunity probably accounts for the milder clinical and histological phenotype.

The diagnosis of IL-12R $\beta$ 1 deficiency in patients with the mild form of the syndrome is based principally on the detection of the  $\beta$ 1 chain on activated T cell PHA-blasts by flow cytometry with specific antibodies. Functional assays concern the IL-12 enhancement of the destruction of K562 cells by NK cells or IFN $\gamma$  production by blood cells stimulated with IL-12 alone or IL-12 plus another stimulus. Genetic studies are often necessary to confirm the diagnosis. Patients are treated with antibiotics and, if appropriate, IFN $\gamma$ . As with the other deficiencies, the dose of IFN $\gamma$  may be optimized individually, with side effects remaining tolerable. In our experience, the treatment of abdominal lesions is difficult and does not respond very well to even high doses of IFN $\gamma$ . Surgery to remove enlarged spleen or abdominal lymph nodes may be of benefit for the patients, improving their condition. Bone marrow transplantation is not indicated. As with other genetic diseases underlying severe mycobacterial infections it is unknown whether prophylactic treatment with antibiotics, IFN $\gamma$ , or both is of benefit, although most patients with IL-12R $\beta$ 1 deficiency do not currently receive such prophylaxis.

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## Conclusion

Idiopathic disseminated infections due to BCG or NTM have long been suspected to be due to a Mendelian genetic disorder. In the past five years, this prediction has been confirmed and different types of mutation in five genes, *IFNGR1*, *IFNGR2*, *STAT1*, *IL12B*, and *IL12RB1*, have been identified. The nine disorders resulting from these mutations are genetically different but immunologically similar as impaired IFN $\gamma$ -mediated immunity is the common pathogenic mechanism accounting for mycobacterial infection in all patients. The severity of the histological and clinical phenotype depends on the type of genetic defect. Complete IFN $\gamma$ R1 and IFN $\gamma$ R2 deficiencies predispose patients to overwhelming infections with impaired granuloma formation in early childhood, whereas partial IFN $\gamma$ R1, IFN $\gamma$ R2 and STAT-1 deficiencies and complete IL-12 p40 and IL-12R $\beta$ 1

deficiencies predispose patients to curable infections with mature granulomas at various ages.

The discovery of these genetic disorders has important diagnostic and therapeutic implications. Diagnosis remains challenging because the syndrome is heterogeneous and there are few simple standardized assays. Tests to detect IL-12 and IFN $\gamma$  cytokines and their receptors with specific monoclonal antibodies are often followed by functional assays aimed at determining the cellular response to IL-12 and IFN $\gamma$ . Gene sequencing and gene transfer, guided by biochemical and functional assays, provide the definitive diagnosis in most cases.

An accurate molecular diagnosis is indeed crucial to determine the optimal treatment strategy for individual patients. Antibiotics should not be discontinued and bone marrow transplantation

may be considered in children with complete IFN $\gamma$ R1 or IFN $\gamma$ R2 deficiency, in whom IFN $\gamma$  treatment is ineffective and mycobacterial infections overwhelming. In children with partial IFN $\gamma$ R1, IFN $\gamma$ R2, and STAT-1 deficiencies, antimycobacterial drugs may be sufficient but IFN $\gamma$  therapy may also be of benefit. Likewise, antibiotics and IFN $\gamma$  treatment are likely to be effective in patients with complete IL-12 p40 or IL-12R $\beta$ 1 deficiency. In contrast, the treatment of patients with severe BCG and NTM infections but no known genetic defect remains empirical. Future research will focus on the search for other underlying genetic defects in order to provide a rational basis for the diagnosis and management of patients with mycobacterial disease.

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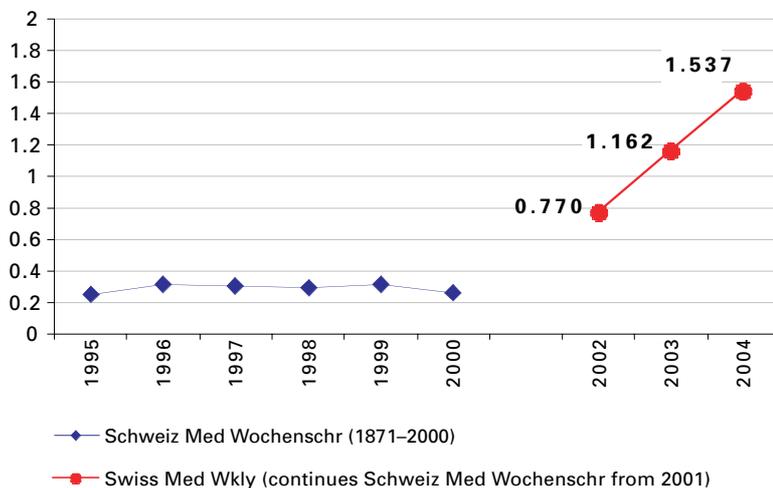
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