El síndrome de inmunodeficiencia primaria descrito recientemente y denominado susceptibilidad mendeliana a las enfermedades micobacterianas (MSMD, MIM 209950), se caracteriza por infecciones diseminadas causadas por micobacterias poco virulentas, *Salmonella* y/o *Mycobacterium Tuberculosis*, en individuos, por lo demás, sanos. El análisis molecular de las familias afectadas ha permitido identificar mutaciones en cinco genes del eje IL-12/IFN-γ, destacando la importancia de esta vía en la inmunidad humana frente a las micobacterias. La heterogeneidad genética explica el amplio espectro clínico de la MSMD que abarca desde las mutaciones en los genes IFNGR1 o IFNGR2, con un defecto completo de receptor e infecciones diseminadas y fatales en la infancia, hasta el otro extremo, en el que se han hallado mutaciones en los genes IFNGR1, STAT1, IL-12 e IL-12Rβ1 en individuos que no han padecido infecciones por micobacterias ni salmonella. También se ha podido observar que existe una correlación entre el fenotipo clínico y los hallazgos histopatológicos. La función inmunológica en los pacientes con MSMD es en general normal. Los defectos hereditarios del eje IL-12/IFN-γ deberían considerarse en el diagnóstico diferencial de todos los pacientes con infecciones severas por microorganismos intracelulares, especialmente cuando éstos se consideran no patógenos en el individuo inmunocompetente. Los niveles plasmáticos de IFN-γ, la expresión de la proteína, los estudios funcionales y en última instancia el análisis del DNA, son procedimientos diagnósticos, pero en aproximadamente la mitad de los pacientes con MSMD no se han hallado mutaciones en IFNGR1, IFNGR2, STAT1, IL-12B o IL-12RB1. Los niños con MSMD deberían ser tratados a nivel individual, en colaboración con algún centro especializado en el manejo de estos pacientes. Es necesario investigar un número mayor de pacientes para poder entender mejor la inmunidad micobacteriana humana.

**PALABRAS CLAVE:** BCG / Infecciones por micobacterias / INF-γ / IL-12.
EM infection(5-7). et al reported 3 male members of one family with disseminated 2 siblings with reported at around the same time(10,11). The fact that patients and families with more than one affected member were first vaccination was first reported in 1951 (8). A sporadic case first cousin of this child also had disseminated BCG infection(14). absence of recognised immunodeficiency are well described genes await discovery. has yet to be identified, suggesting that mutations in other remains a number of patients for whom a genetic aetiology of this pathway in human immunity to mycobacteria. There included various environmental mycobacteria (EM), and virulent mycobacteria in individuals without recognised reported cases of severe disseminated infection with weakly- INTRODUCTION Over the past 50 years there have been a number of reported cases of severe disseminated infection with weakly-virulent mycobacteria in individuals without recognised predisposing immunodeficiency. Mycobacterial species included various environmental mycobacteria (EM), and several M. bovis BCG vaccine substrains. High rates of affected siblings and parental consanguinity suggested the existence of a novel primary immunodeficiency syndrome, subsequently named Mendelian Susceptibility to Mycobacterial Disease (MSMD, MIM 209950). Molecular investigation of these families has identified mutations in five genes in the IL-12-dependent IFN- of these families has identified mutations in five genes in the IL-12-dependent IFN- axis highlighting the importance of this pathway in human immunity to mycobacteria. There remain a number of patients for whom a genetic aetiology has yet to be identified, suggesting that mutations in other genes await discovery. Sporadic cases of disseminated EM infection in the absence of recognised immunodeficiency are well described(13). Familial disseminated EM infection was first reported in 1964: three members of the same Danish family had fatal disseminated M. avium complex (MAC)(3). Uchiyama identified 2 siblings with M. avium infection(4). More recently, Holland et al reported 3 male members of one family with disseminated EM infection(18). Idiopathic disseminated BCG infection following vaccination was first reported in 1951(5). A sporadic case born to consanguineous parents was described in 1973(6), and families with more than one affected member were first reported at around the same time(7,8). The fact that patients with inherited susceptibility to mycobacterial infections may also be at increased risk of Salmonella infection was first highlighted in a report by Heyne who described a brother and sister from Germany who developed generalised infection after neonatal BCG vaccination(9). The boy later a 3 year old boy who had been vaccinated with BCG at the age of 3 days, developed disseminated Salmonella and BCG infection resulting in his death 3 years later(10). A first cousin of this child also had disseminated BCG infection(11). The first family in which the molecular basis of increased susceptibility to EM was elucidated was described in 1995(12). Four children from the same village in Malta all developed disseminated EM infection. Two were brothers related to a third child as fourth cousins, whilst the fourth child was not knowingly related to the others. The parents of the brothers were second cousins and both related to both parents of the fourth cousin. Each child was infected with a different mycobacterial species (M. chelonae, M. fortuitum and two different stains of MAC), suggesting an innate defect in host immunity was responsible. However, extensive immunological investigation failed to identify any known defect predisposing to such infections. Patients had defective upregulation of monocyte function in response to endotoxin and IFN-γ(13) and defective antigen presentation(14). The high degree of consanguinity within the Maltese family suggested the children were homoygous for a rare recessive mutation inherited from a common ancestor. A whole genome search for homozygosity in three of the affected children mapped the gene to the region of chromosome 6q containing the gene encoding the IFN-γ receptor ligand binding chain (IFN-γR1) of the IFN-γ receptor complex(15). A mutation in the coding region of this gene (IFNGRI), which resulted in complete absence of IFN-γR1 expression at the cell surface, was subsequently identified as the cause of the defect(15). Meanwhile, a survey conducted in parallel to the work described above found that of 108 cases of disseminated infection following BCG vaccination reported since 1951, 50% were idiopathic(16). A retrospective study of all cases of disseminated BCG infection following vaccination in France between 1974 and 1994 revealed that out of 32 children identified, 16 had no recognised predisposing immunodeficiency(17). Among a total of 60 children worldwide with idiopathic disseminated BCG infection for whom information was available, four pairs of siblings and one pair of first cousins were identified, and parental consanguinity was noted in 24 families. Clinical and histopathological features in a Tunisian child with disseminated BCG infection, born to consanguineous parents, were remarkably similar to those of the Maltese children with EM infections. A series

### TABLE I. Genes involved in defective macrophage activation

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene product</th>
<th>Chromosomal location</th>
<th>MIM number</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNGRI</td>
<td>Interferon-γ receptor ligand binding chain</td>
<td>6q23-q24</td>
<td>1079470</td>
</tr>
<tr>
<td>IFNGR2</td>
<td>Interferon-γ receptor signal transducing chain</td>
<td>21q21.1-22.2</td>
<td>145639</td>
</tr>
<tr>
<td>IL-12RB1</td>
<td>Interleukin-12 receptor beta-1 subunit</td>
<td>19p13.1</td>
<td>601604</td>
</tr>
<tr>
<td>IL-12B</td>
<td>Interleukin-12 receptor beta-2 subunit</td>
<td>5p13.1-33.1</td>
<td>161561</td>
</tr>
<tr>
<td>STAT1</td>
<td>Signal transducer and activator of transcription 1</td>
<td>2q12.2-32.3</td>
<td>600555</td>
</tr>
</tbody>
</table>
of candidate genes involved in anti-mycobacterial immunity in the mouse model of BCG infection was tested by homozygosity mapping. The segregation of markers within IFNGR1 suggested linkage and a frameshift mutation resulting in the absence of IFNγR1 expression was identified (20).

Although mutations in IFNGR1 were subsequently identified in other cases of MSMD, there were a number of patients in whom mutations within IFNGR1 were not detected. Investigation of other candidate genes within the IFN-γ pathway led to the identification of mutations in four other genes (Table I), all of which are involved in the IL-12-dependent IFN-γ-mediated immunity reviewed in (21-23).

### TABLE II. Clinical features of MSMD according to gene involved

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Infection</th>
<th>RES</th>
<th>Bone</th>
<th>CNS</th>
<th>GIS</th>
<th>RS</th>
<th>Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>cIFN-γR1</td>
<td>MAC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. fortuitum</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td></td>
<td>M. chelonae</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>BCG</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>M. oxynitri</td>
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<td>+</td>
<td></td>
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<tr>
<td></td>
<td>M. kansasii</td>
<td>+</td>
<td>+</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>M. szulgai</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>Salmonella</td>
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</tr>
<tr>
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<tr>
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<tr>
<td></td>
<td>MAC</td>
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<td></td>
</tr>
<tr>
<td>AR pIFN-γR1</td>
<td>BCG</td>
<td>+</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella</td>
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<td></td>
</tr>
<tr>
<td>AR pIFN-γR2</td>
<td>BCG</td>
<td>+</td>
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<td></td>
<td>M. abcessus</td>
<td>+</td>
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</tr>
<tr>
<td>AD pIFN-γR1</td>
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</tr>
<tr>
<td></td>
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<td>+</td>
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<tr>
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<tr>
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<td>M. kansasii</td>
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<tr>
<td></td>
<td>Salmonella</td>
<td>+</td>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
<td></td>
<td>M. tuberculosis</td>
<td>+</td>
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</tr>
<tr>
<td></td>
<td>Salmonella spp</td>
<td>+</td>
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<tr>
<td></td>
<td>Norcardia asteroides</td>
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<tr>
<td>cIL-12Rβ1</td>
<td>BCG</td>
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<tr>
<td></td>
<td>MAC</td>
<td>+</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>M. fortuitum-chelonae</td>
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</tr>
<tr>
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<td>M. tuberculosis</td>
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<tr>
<td></td>
<td>Salmonella</td>
<td>+</td>
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<td></td>
</tr>
<tr>
<td>STAT1</td>
<td>BCG</td>
<td>+</td>
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</tr>
<tr>
<td></td>
<td>MAC</td>
<td>+</td>
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</tr>
</tbody>
</table>

| cIFN-γR, complete IFN-γR deficiency; AR pIFN-γR, autosomal recessive partial IFN-γR deficiency; AD pIFN-γR, autosomal dominant partial IFN-γR deficiency; cIL-12p40, complete IL-12p40 deficiency; cIL-12Rβ1, complete IL-12Rβ1 deficiency; STAT1, partial signal transducer and activator of transcription 1 deficiency; RES, Reticuloendothelial system; CNS, Central nervous system; GIS, Gastrointestinal system; RS, Respiratory system; BCG, bacille Calmette-Guérin; MAC, Mycobacterium avium complex.

### CLINICAL AND PATHOLOGICAL MANIFESTATIONS

The central feature of MSMD is infection with weakly-pathogenic mycobacteria. In keeping with the genetic heterogeneity there is a clinical spectrum of MSMD. At one end of the spectrum, mutations in IFNGR1 or IFNGR2, which result in a lack of functional protein at the cell surface
INHERITED DISORDERS OF THE INTERLEUKIN-12/INTERFERON-GAMMA AXIS... VOL. 22 NUM. 3/ 2003

...have a very poor prognosis, with the development of disseminated infection in early childhood and progressively fatal disease(20,24,17). At the other end, screening of family members has identified individuals who carry mutations involving IFNGR1, STAT1, IL-12B and IL-12RB1, who have not developed infection with either mycobacteria or salmonella(25-28). Other IFNGR1 and IFNGR2 mutations resulting in the expression of an abnormal protein causing partial receptor deficiency are associated with milder phenotypes and response to IFN-γ treatment(27,29,30). Similarly, mutations in the genes encoding the IL-12 p40 subunit (IL-12B) or the IL-12 receptor β1 subunit (IL-12RB1), resulting in complete deficiency of either protein, result in a less severe phenotype and good response to antimicrobial and IFN-γ treatment. The signal transducer and activator of transcription 1 (STAT1) mutation reported is phenotypically similar to partial IFN-γR deficiency(31).

A striking feature of MSMD is the specific susceptibility to poorly-pathogenic mycobacterial species. Various mycobacteria species have been isolated including slow-growing species, such as M. kansasii, M. avium and M. szulgai and rapid-growing species, such as M. smegmatis, M. abscessus, M. chelonei, M. fortuitum, and M. peregrinum. M. smegmatis(32) and M. peregrinum (Koscietlnak et al., submitted) had not previously been documented as causes of disseminated EM disease. The more virulent M. tuberculosis has been implicated in or isolated from individuals with IFN-γR1, IL-12p40 and IL-12RB1 deficiencies(25,28,39-43). The mycobacterial species identified correlate with the genetic defect. For example, rapid-growing mycobacterial species are mostly observed in children with complete IFN-γR1 or IFN-γR2 deficiency (Table II). Salmonella infections ranging from protracted gastroenteritis to septicemia and disseminated infection occurred in about a quarter of reported cases, more commonly in association with IL-12p40 and IL-12RB1 deficiencies. Other pathogens isolated from MSMD cases include Listeria monocytogenes(33), Histoplasma capsulatum(27) and Norcardia asteroides(34). Fungal and bacterial pathogens such as candida and staphylococci have not caused infection, despite the presence of indwelling intravenous catheters in many patients. Increased susceptibility to viral infections, particularly with herpes viruses, has been noted in some patients in whom MSMD has been shown to be due to IFN-γRI deficiency(25,28,30). However, this is not universal and most other patients have had classical childhood viral infections without problems. Mutation of STAT1 has not resulted in increased susceptibility to viral infection, despite the role of STAT1 in both IFN-γ and IFN-α mediated immunity (see section 4).

Patients with MSMD due to complete IFN-γR1 deficiency may present in childhood with a characteristic syndrome of chronic fever, weight loss, lymphadenopathy, hepatosplenomegaly and evidence of disseminated infection which may involve bone, skin, soft tissues, lung and meninges. The clinical presentation appears to vary according to the genetic defect involved. For example, dominant partial IFN-γR1 deficiency is almost always associated with osteomyelitis(37,38), while lymphadenopathy is a very common feature of IL-12p40 or IL-12RB1 deficiency(25,30-32). The clinical features of each genetic defect remain to be carefully described (Dorman and Picard in progress). The age of onset varies according to the gene involved, the type of mutation and whether the affected individual received BCG vaccination at birth or acquired EM infection via natural routes.

![Figure 1. Granulomata type 1 and type 2. Left, hematoxilin and eosin staining of granulomata type 1 lesions (x100). Right, Ziehl-Neelson staining of granulomata type 2 lesions (x400).](image-url)
Correlation between clinical phenotype and histopathological findings has been observed\(^{(44)}\). Two distinct histological types have been documented which appear to be associated with distinct clinical phenotypes (Fig. 1). Approximately half of the patients with disseminated BCG infections had tuberculoid (type I) granulomata with well-defined epithelioid and giant cells surrounded by lymphocytes and fibrosis containing only occasional acid-fast bacilli. The remaining patients had lepromatous-like (type II) lesions with poorly-formed granulomata containing large numbers of acid-fast bacilli. Patients with type I granulomata had a good prognosis but virtually all the children with poor granuloma formation (type II) died. EM granulomata tend to be poorly-formed irrespective of the clinical outcome and underlying genetic defect.

LABORATORY FINDINGS

Chronic infection leads to normochromic, normocytic anaemia, and raised inflammatory markers. Immune function has been extensively investigated in an attempt to identify a known immunodeficiency, and is in general remarkably

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Figure 2. Mutations identified to date in the 5 MSMD genes. The gene-coding regions are indicated with vertical bars separating the exons, designated by roman numerals. Mutations in italic (nonsense and splice mutations and frameshift insertions and deletions; recessive) cause complete deficiency with no detectable protein expression at the cell surface. Mutations in bold (missense mutations and in-frame deletions; recessive) cause complete deficiency with detectable surface protein expression. Mutations in bold italic (dominant) cause partial deficiency.
normal. CD4 T helper cells are often normal but may be low secondary to chronic infection. Levels of serum immunoglobulin isotypes, including IgG subclasses, are normal or elevated, and antigen-specific antibody titres are normal. T-cell proliferation in vitro, in response to various mitogens and recall antigens, are also normal. Polymorphonuclear cells are normal in terms of morphology, CD18 expression, chemotaxis, and respiratory burst. Delayed type hypersensitivity (DTH) testing in vitro and blastogenesis in vitro to PPD are normal in patients with complete IFN-γR1 and IL-12Rβ1 deficiency, indicating that IL-12 and IFN-γ are not required for DTH or blastogenesis to mycobacterial antigens.

**MOLECULAR BASIS OF THE DISEASE**

Mutations in 5 genes of the IL-12/IFN-γ axis causing increased susceptibility to mycobacteria have been identified to date (summarised in Fig. 2). This pathway is central to the immune response to intracellular pathogens such as mycobacteria and the functions of these genes are described in more detail in section 5.

**IFNGR1**

Mutations in this gene were the first to be identified as the cause of MSMD (26,27). Subsequent investigation of patients with increased susceptibility to poorly pathogenic mycobacteria has led to the identification of at least 15 null recessive mutations in this gene (Fig. 2) (28-34,36-38). The identification of families in which mycobacterial infections occurred in more than one generation suggested that dominant mutations might also exist (39). Investigation of 18 individuals from 12 kindreds led to the identification of a small deletion hotspot within IFNGR1. A 4 base pair deletion at nucleotide position 818 (818del4) was identified in 11 of the unrelated kindreds and the 12th family had a single nucleotide deletion (T) in this position (818delT). The 818del4 mutation leads to a premature stop codon within the intracellular domain of the receptor. The receptor is expressed on the cell surface but the mutant receptor lacks the three motifs required for intracellular signalling (the JAK1 and STAT1 binding sites, and the tyrosine phosphorylation site). It also lacks a recycling motif so the truncated receptor accumulates on the cell surface and interferes with signaling by the normal receptor encoded by the normal copy of IFNGR1. Thus the mutant allele has a dominant effect (in comparison to the recessive form of IFNGR1 deficiency, where parents are healthy carriers of the mutations). Subsequently, other IFNGR1 mutations resulting in a dominantly inherited phenotype have been identified (39,40). A second small deletion hotspot was recently identified in IFNGR1, in this case with a recessive phenotype (40,41). In summary, a range of mutations including frameshift, insertion, deletion, nonsense, missense and splice mutations have been identified in IFNGR1 (Fig. 2). All recessive mutations identified to date occur in the part of the gene encoding the extracellular domain of the receptor chain, the majority of which result in complete lack of receptor expression. Two of the recessive mutations allow expression of a poorly-functioning protein leading to partial deficiency (42). Partial receptor deficiency may also result from dominant mutations leading to a receptor deprived of its intra-cyttoplasmic segment.

**IFNGR2**

Complete deficiency of IFN-γR2 was found in a child with disseminated M. fortuitum and MAC infections in whom cell surface expression of IFN-γR1 and IFN-γR3 sequence were normal (43). Sequence analysis of IFNGR2 led to the identification of a 2 base pair deletion (277del2), which in turn led to a premature stop codon. The truncated protein lacked both the transmembrane and intracellular (signalling) domains and was not expressed at the cell surface. Both parents, though unrelated, carried this mutation for which the child was homozygous. A case of partial IFN-γR2 deficiency has also been described (44). A child born to related Portuguese parents developed disseminated infection following BCG vaccination. At the age of 16 she developed M. abscessus infection. A point mutation was identified in the IFNGR2 sequence: the patient was homozygous for the mutation while both parents were heterozygous. This mutation results in an amino acid substitution at position 141 (arginine-cysteine) within the extracellular domain. The mutant IFN-γR2 is expressed normally on the cell surface.
but presumably the affinity between IFN-γR1 and IFN-γR2 is impaired.

**IL-12B**

Complete IL-12 p40 subunit deficiency leading to MSMD and Salmonella infections has also been described. The first case was born to consanguineous Pakistani parents and was immunised with BCG at birth\(^{(42)}\). Sequestration of IL-12B revealed a deletion involving two coding exons. The parents and a healthy sibling were carriers of this mutation: the affected child was homozygous. Eleven other patients from five other families have recently been identified\(^{(25,39,40,43,51,52)}\). One child had only salmonellosis. All other patients had mycobacterial disease: BCGosis in 10 children and *M. chelonei* in one child. Four children with BCGosis also had salmonellosis, one had tuberculosis, and one had nocardiosis. Five children died but all survivors are well off all treatment. Interestingly, one kindred from India had the same large deletion previously reported in the Pakistani kindred. A founder effect was documented and dated to approximately 29 generations ago (95% CI 9-113) and 700 years ago (95% CI 216-2,760) using a novel mutation dating method (Abel L. et al. submitted). The other four kindreds originated from the Arabic peninsula and were all found to carry the same IL-12B frameshift insertion. A founder effect was again documented, and dated to 87 generations ago (95% CI 22-110) and 1,100 years ago (95% CI 528-2,640). The fact that all patients with IL-12p40 deficiency identified to date have IL-12B mutations resulting from a founder effect, one in the Indian subcontinent and another in the Arabic peninsula, is consistent with the rarity of IL-12p40 deficiency among patients with MSMD. It is the first example of a founder effect among mendelian mycobacterial susceptibility genes. IL-12 p40 (IL-12B) has recently been shown to be a subunit of IL-23. Thus IL-12 p40 deficiency probably results in IL-23 deficiency: however owing to the lack of human IL-23 specific antibodies, this cannot be ascertained as yet.

**IL-12RB1**

Mutations in IL-12RB1, which encodes the β1 subunit of the IL-12 receptor, have been identified in seven patients from eight different families with EM infection to date\(^{(25,39,40,43,51,52)}\). Five of these patients also had Salmonella infections. A total of 8 mutations have been identified to date, including nonsense, splice and frameshift mutations which lead to premature termination of translation in the extra cellular domain. This abrogates cell surface expression resulting in complete IL-12RB1 deficiency. Two missense mutations, also resulting in a lack of receptor expression at the cell surface, were recently validated by gene transfer\(^{(25,40)}\). 

Only recessive, loss-of-function mutations have been identified in IL-12RB1 to date. Recently, a series of 31 patients from 23 kindreds with IL-12RB1 deficiency has been described\(^{(25)}\). Most patients had BCG/NTM disease, often with salmonellosis, but several were found to suffer from salmonellosis only and some from tuberculosis only. A significant fraction of patients were strictly asymptomatic. The recent observation that IL-12RB1 also serves as a subunit in the IL-23 receptor suggests that IL-12RB1 mutations prevent IL-23 activation, but this has not been experimentally tested as yet.

**STAT1**

The identification of two unrelated families presenting with MSMD in the absence of mutation in any of the above genes led recently to the discovery of the fifth MSMD gene. A 33-year-old woman with a history of disseminated BCG infection following childhood vaccination, and a 10-year-old girl with disseminated *M. avium* infection were found to carry a *de novo* mutation in the coding region of STAT1\(^{(26)}\). Both were heterozygous for a single T-C nucleotide change at position 2116 resulting in L706S at the COOH terminal region. The abnormal protein exerts a dominant negative effect on the normal protein in terms of STAT-1 dimer (also known as gamma activating factor, GAF) activation, but not in terms of STAT-1/STAT-2/p48 trimer (also known as interferon-stimulated gamma factor 3, ISGF3) activation. The STAT1 mutation is loss-of-function for the two cellular phenotypes (it impairs phosphorylation of tyrosine 701) but dominant for one (GAF activation) and recessive for another (ISGF3 activation) in heterozygous cells stimulated with either type of IFN. It is, to our knowledge, the first reported mutation in a human gene to be dominant and recessive for two cellular phenotypes. Vulnerability to mycobacteria and resistance to viruses in the patients thus imply that GAF mediates anti-mycobacterial IFN-γ activity, whereas the anti-viral effects of IFNs are either STAT-1 independent or ISGF3 dependent. This novel disorder proves that IL-12-induced IFN-γ mediated immunity against mycobacteria is both STAT1 and GAF-dependent.

**FUNCTIONAL ASPECTS OF THE PROTEINS**

The receptor for IFN-γ consists of two subunits: IFN-γR1, the ligand-binding chain (previously known as the α chain) and IFN-γR2, the signal-transducing chain (previously known as the β chain or accessory factor-1)\(^{(34)}\). As the ligand-binding chains interact with IFN-γ homodimers, they dimerise and become associated with two signal-transduction chains. This leads to the activation of specific members of two protein families: the Janus kinases (JAK) and the signal-
Clinical diagnosis

Inherited defects of IL-12/IFN-γ axis may be considered in the differential diagnosis of all patients presenting with severe infection (including disseminated and recurrent diseases) with intracellular microorganisms, particularly when the organism is considered to be non-pathogenic in the "immunocompetent" individual. However, these defects should be sought aggressively in patients with severe nontuberculous mycobacterial or salmonella infections. Furthermore, a high index of suspicion is warranted in patients presenting with chronic fever, wasting, hepatosplenomegaly, lymphadenopathy, and anaemia in whom a pathogen is not isolated, as cultures may be persistently negative. Diagnosis may also be confounded by the lack of usually diagnostic granulomata, in which microbes may or may not be visible. An initial diagnosis of histiocytosis X has occasionally been made, hence MSMD should be considered in chemotherapy-resistant children with a tentative diagnosis of histiocytosis without formal histological criteria.

In many individuals, MSMD becomes apparent following BCG vaccination and vaccination history is therefore essential. In vitro diagnosis

Circulating IFN-γ levels

Measurement of circulating IFN-γ in either plasma or serum is a simple means to differentiate patients with complete IFN-γR deficiency from those with other MSMD mutations. These children have high levels of plasma IFN-γ whereas IFN-γ is low or undetectable in plasma taken from healthy controls, or MSMD patients with IL-12p40 or IL-12Rβ1 deficiency or partial IFN-γR1 or IFN-γR2 deficiency. This is thought to be due to sustained production of IFN-γ in the most severe form of MSMD and/or the requirement for an intact IFN-γR for ligation and removal of IFN-γ from the circulation. This observation provides a simple diagnostic assay for individuals presenting with severe BCG/EM disease. However, it should be kept in mind that elevated plasma or local (e.g., pleural tuberculosis) IFN-γ levels may also be seen in more normal hosts with tuberculosis.

Strategies for diagnosis

Clinical diagnosis

Inherited defects of IL-12/IFN-γ axis may be considered in the differential diagnosis of all patients presenting with severe infection (including disseminated and recurrent diseases) with intracellular microorganisms, particularly when the organism is considered to be non-pathogenic in the "immunocompetent" individual. However, these defects should be sought aggressively in patients with severe nontuberculous mycobacterial or salmonella infections. Furthermore, a high index of suspicion is warranted in patients presenting with chronic fever, wasting, hepatosplenomegaly, lymphadenopathy, and anaemia in whom a pathogen is not isolated, as cultures may be persistently negative. Diagnosis may also be confounded by the lack of usually diagnostic granulomata, in which microbes may or may not be visible. An initial diagnosis of histiocytosis X has occasionally been made, hence MSMD should be considered in chemotherapy-resistant children with a tentative diagnosis of histiocytosis without formal histological criteria.

In many individuals, MSMD becomes apparent following BCG vaccination and vaccination history is therefore essential.
cells stimulated with PDBU. To date, all cells stimulated by BCG, or in the supernatant of EBV-B p70 can be detected by ELISA in the supernatant of blood and SV40-transformed fibroblasts. Secreted IL-12p40 and well: this is not the case for other cell lines such as EBV-B Staining of identified cause a loss of expression of the encoded chain.

12R
12p40 and IL-12p70. However, prolonged stimulation of IL-
identified have been associated with a lack of detectable IL-
γ
of IL-12R

12 deficiency may be diagnosed functionally by studying in vitro mobility shift assays(36, 54), or more simply by flow cytometry, using a STAT1 specific monoclonal antibody(60). Cellular responses to IFN-γ should be tested at low and high concentrations of IFN-γ to differentiate between partial and complete receptor deficiency. In vitro studies in patients with IL-12 p40 deficiency show defective IFN-γ production by PBMC or whole blood following stimulation with BCG. This defect was restored by the addition of recombinant IL-12 to the culture medium. IL-12RB1 deficient patients also have diminished mitogen-induced IFN-γ production, but IL-12 p70 production in response to LPS, tuberculin or mycobacteria is normal. A flow cytometric assay which detects phosphorylated STAT1 has also been developed(30). Notably, children with complete IFN-γR deficiency have a low in vitro production of IFN-γ, due to impaired production of IL-12(40).

DNA analysis
With the exception of the dominant IFNGR1 hotspot mutations and the two IL-12B (p12-p40) mutations (founder effects), every MSMD family has a unique mutation. It is therefore not cost-effective to set up mutation screening assays looking for known mutations. A combination of in vitro phenotyping (expression and functional studies) and direct gene sequencing is recommended. Once the causative mutation has been established in a family, other family members can be screened directly for the mutation. Accurate molecular diagnosis by biochemical, functional, and genetic studies is of the utmost importance for predicting clinical outcome and guiding the treatment of patients.

GENETIC COUNSELLING AND PRENATAL DIAGNOSIS
Defects in the IL-12/IFN-γ pathway may be inherited either as dominant or recessive disorders depending on the mutation. All mutations reported in IFNGR2, IL-12RB1 and IL-12B are recessive: many patients are homozygous for recessive mutations, reflecting the high frequency of parental consanguinity within this group of patients. IFNGR1 mutations were initially identified as homozygous recessively inherited, but dominant mutations have subsequently been identified as well. Compound heterozygotes have also been identified. The STAT1 mutation identified in three individuals has been suggested in one kindred, though the molecular basis of increased susceptibility to EM in this family has yet to be established(5,7).

Given the heterogeneity of this syndrome, coupled with its rarity, carrier detection/screening is not currently feasible. In one family with recessive IFN-γR1 deficiency, heterozygous carriers had an intermediate cellular phenotype in vitro(17) although this may have been dependent on the assay used. To date, there is no clinical phenotype associated with heterozygosity for any of the recessive alleles. Once the molecular basis is known within a family, it is simplest to
screen other members by looking directly at their DNA. Counselling within families where the mutation is known is straightforward in terms of the risk of inheriting a «susceptible» genotype (25% risk of an affected child if recessive, 50% risk of an affected child if dominant inheritance). However, any discussion must also take into account the following: a) the clinical phenotype depends on the gene affected and whether the mutation leads to complete or partial protein deficiency; b) the development of disease is dependent on pathogen exposure; c) there are individuals with less severe mutations involving either IFNGR1 or IL-12Rβ1 who have inherited a susceptible genotype but have not developed disease. Presumably they have some residual antimycobacterial immune function. To date, there are no known patients with complete IFN-γR1 or IFN-γR2 deficiency who have not been affected.

Complete IFN-γR1 or IFN-γR2 deficiency is the most severe phenotype and is frequently lethal despite antibiotics. BCG vaccination must be withheld from potentially affected children until IFN-γR status is clarified. Bone marrow transplantation has proved very difficult, and less successful than would be anticipated (see below) perhaps because transplantation has typically been attempted after disseminated mycobacterial disease has occurred.

Once a molecular diagnosis has been established, prenatal diagnosis can be offered to affected families with severe disease, i.e. complete IFN-γR deficiency. The role of prenatal diagnosis for other mutations is less obvious as the phenotype is less severe, disease is preventable and many individuals carrying mutations are disease-free.

TREATMENT AND PROGNOSIS

The treatment of defects in the IL-12/IFN-γ axis should be tailored to the individual patient according to their mutation, the clinical pattern of disease and the pathogens involved[61]. Established infection should be treated with appropriate antimicrobial drugs as determined by the genus and species. Thus microbiological isolation and characterisation of the causative pathogen at an early stage is desirable. The role for in vitro susceptibilities in directing treatment of EM is still unproven and poorly defined. EM are notoriously resistant to a number of antimicrobials. Cytokine therapy has helped clear mycobacterial infection in patients with full or partial function of the IFN-γR[62]. Patients with IL-12B (IL-12p40) or IL-12Rβ1 deficiency, or partial IFN-γR deficiency respond well to IFN-γ treatment. However, intestinal/mesenteric/splenic infections can be resistant to antibiotics and IFN-γ. Splenectomy was helpful in two children with splenic sequestration (IFN-γ induced in one child); on occasions abdominal lymph node resection may be indicated[61] (Casanova JL, unpublished). Overall, patients with partial IFN-γR/STAT1 deficiency or complete IL-12Rβ1 deficiency can achieve prolonged clinical remission after antibiotics and IFN-γ are discontinued. Relapses may occur years after the initial episode. Treatment with antibiotics and IFN-γ should be prolonged, even after clinical remission is obtained.

In contrast, children with complete IFN-γR deficiency achieve full clinical remission less often and mycobacterial infections often relapse weeks to months after antibiotics are discontinued. Therefore, successful antibiotic therapy should not be discontinued. Due to lack of specific receptors, IFN-γ therapy is not indicated. The role for other cytokines such as IFN-α, GM-CSF or IL-12 is undefined. The only curative treatment available for patients with complete IFN-γR deficiency is bone marrow transplantation (BMT). An international survey of 11 patients who underwent BMT is currently in progress[61]. Preliminary results indicate that BMT in patients with complete IFN-γR deficiency is associated with an unexpectedly high level of post-BMT morbidity and mortality. The only child who received an HLA-haplo-identical transplant rejected the graft. Of nine unrelated patients who received an HLA-identical intrafamilial graft, despite an initial full engraftment in all cases, the graft was secondarily rejected in six children, four of whom have died. Therefore, there appears to be a selective advantage of IFN-γR-deficient over wild-type haemopoietic progenitors in IFN-γR-deficient children. This makes gene therapy for bone marrow IFN-γR deficiency challenging, as a selective advantage of transduced cells is absolutely required. Prevention of infection is desirable, although many pathogens to which these individuals are susceptible are ubiquitous in the environment. BCG should be avoided and mycobacterial infection (both primary and secondary) may be prevented by the use of a macrolide such as clarithromycin or azithromycin. In patients with mild MSMD, prophylactic antibiotics are not absolutely required, as infectious episodes are relatively infrequent and can be controlled by IFN-γ and antibiotics if treated promptly. However, physicians and patients should weigh carefully the risks and benefits of recurrence of infection, especially if it recurs in bone, as is the case often with the dominant form of IFN-γR1 deficiency. In these patients, recurrence of infection can have serious consequences, despite curative therapy.

In patients with complete IL-12R deficiency, antibiotics should be continued indefinitely, after therapy of acute infections. There is considerable diversity of pathogenic EM (particularly rapidly-growing species), making absolute recommendations difficult. However, most EM are susceptible to macrolides, and these should be strongly considered for...
long-term prophylaxis regardless of cure of other acute infections. Immunosuppression such as corticosteroids should be avoided as a rule, particularly in children with complete IFN-γ deficiency, although in some circumstances they may be helpful. Children with MSMD should be treated on an individual basis, and treatment undertaken in close collaboration with a centre specialised in the care of such patients.

**ANIMAL MODELS**

The study of gene disrupted mice has greatly enhanced our understanding of the IL-12/IFN-γ pathway. Although not completely concordant, the phenotypic similarities between these animal models and patients with mutations in this axis are striking(60). Mice lacking Ifng1 are highly susceptible to BCG infection, with poorly defined granuloma formation and death(60). Mice lacking Ifng also fail to control BCG, M. avium or M. tuberculosis growth(61-63). More recently, Ifng2 knockout mice were shown to have defective IFN-γ production and susceptibility to L. monocytogenes infection(64). Il-12b (IL-12 p40) knockout mice are more susceptible to M. tuberculosis infection than normal mice, leading to higher bacterial loads and disseminated disease(65). Granulomata were poorly formed and multicellular. Il-12b1 knockout mice have defective IFN-γ responses to mitogens and LPS(66). Disruption of Ifng in mice also leads to lethal infection with an attenuated strain of S. typhimurium whereas wild type mice clear infection within four weeks(67, 70). However, comparisons between mouse and man are limited in several ways: most of the infections in MSMD patients are naturally occurring while those in mice are experimental, often administered intravenously, and the strain and dose of pathogen is controlled. There are certain infections, such as Toxoplasma gondii and Cryptococcus neoformans, to which Ifng1/Ifng2 knockout mice have increased susceptibility, which have not been observed in humans(69, 70). This may reflect lack of exposure, experimental design, or the fact that knockout mice are generated in highly inbred strains. Genetic variation at other immunity modifying loci is low in inbred mice whereas humans are outbred, even in the setting of consanguinity. Experimental infections in mice probably highlight even minor effects of the IL-12/IFN-γ axis. Alternatively, mice and humans may be divergent in their handling of some of these non-mycobacterial infections.

**CONCLUDING REMARKS AND FUTURE CHALLENGES**

Mutations in 5 genes involved in the IL-12/IFN-γ axis have been associated with the syndrome of MSMD, which encompasses a range of clinical phenotypes. The severity of the clinical phenotype primarily depends on the gene involved and the specific mutation. IFN-γ mediated immunity appears to be a genetically controlled quantitative trait that determines the outcome of mycobacterial infection(60). IFN-γ immunity to mycobacteria is dependent on IL-12 stimulation and mediated by STAT1 and its homodimeric complex GAβ. These defects are most pronounced with respect to mycobacteria and to a lesser extent salmonella and viruses(70). The investigation of more patients is necessary to broaden our knowledge of these genotype-phenotype correlations. Clinically, molecular diagnosis guides rational treatment based on pathophysiology.

Are there other MSMD genes?

There remain patients with the clinical syndrome of MSMD who do not have mutations in IFNGR1, IFNGR2, STAT1, IL-12B, or IL-12RB1 (approximately 50% at our centres, Holland, Levin and Casanova, unpublished). Characterisation of the molecular defects in these patients will identify other MSMD genes, and contribute further to our understanding of human mycobacterial immunity. Relevant genes upstream of IL-12 and downstream of STAT1 are expected to expand and define the limits of the IL-12/IFN-γ axis, especially the inducer and effector mechanisms of immunity to mycobacteria.

**Definition of the clinical boundaries of MSMD**

The genetic defects of the IL-12/IFN-γ axis were found by investigating patients with disseminated, often lethal, BCG/EM disease. Subsequently, it was found that some affected individuals have recurrent local disease, while others are asymptomatic. International surveys are currently underway to define the clinical features of each inherited disorder, based on the clinical history of the patients identified. The question arises whether patients with unexplained local EM adenitis in childhood, are currently unexplained. Patients with various forms of BCG/EM disease thus need to be explored for the IL-12/IFN-γ axis, in order to define the clinical frontiers of each genetic defect.

What is the role of MSMD genes in susceptibility to tuberculosis and leprosy?

It is estimated that approximately 2 billion individuals worldwide are infected with M. tuberculosis(71). The World Health Organisation estimates there were 8 million new cases of tuberculosis (TB) and 1.9 million deaths from the disease in 1998. The fact that only 10% of individuals infected with M. tuberculosis go on to develop clinical disease suggests that exposure to virulent mycobacteria alone is not sufficient...
and that host immune response is an important determinant of susceptibility (or resistance) to disease(67). Several studies demonstrate a role for host genetic factors as determinants of susceptibility to TB(55). However, the identification of specific genes involved in susceptibility to infectious diseases in outbred human populations is difficult. Complex interactions between the pathogen, which also has a genome, the environment and host factors determine whether an individual is resistant or susceptible to disease. It is likely that a number of genes are involved, but it is not known exactly how many, nor how they interact. Population based studies have reported associations between candidate genes and TB but the effects have been modest and the functional relevance of these findings is yet to be established(68,69).

There is a spectrum of disease within the MSMD syndrome ranging from severe disease which is fatal in early childhood (complete IFN-γR deficiency) to moderate disease in individuals with partial IFN-γR deficiency(70). The IL-12/IL-12Rβ1 mutations have a less severe clinical course. Mutations in IL-12RB1 and IL-12β have been identified recently as a susceptibility factor for the development of abdominal M. tuberculosis infection(71) and tuberculous adenitis(72). Partial deficiency of either IL-12R or IL-12Rβ1 would be expected to have a less severe phenotype than complete deficiency, and to predispose to only more virulent pathogens(73). More subtle polymorphisms in the MSMD genes identified so far could result in impaired expression of a normal protein or normal expression of a slightly altered protein. It is also possible that mutations or polymorphisms in other genes involved in mycobacterial immunity, which have a different role, may cause a different immune defect. Such individuals may retain immunity to organisms of low virulence while remaining susceptible to more virulent species.

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REFERENCES


64. Kaufman HL, Roden M, Nathanson D, Basso TM, Schwartzentruber DJ, Holland SM. Cytokine therapy of mycobacterial infection. Adv Immunol 2000; 70:


