Mendelian susceptibility to mycobacterial infections

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Abstract
Infection due to vaccination with the Calmette-Guerin Bacillus or atypical mycobacterium may occur in patients with no hereditary or acquired immune deficiency. These idiopathic infections affect children or adults that are otherwise healthy (no other opportunistic infections apart from non-digestive salmonellosis). Predisposition to mycobacterium is called Mendelian susceptibility to poorly virulent mycobacteria, such as the bacillus Calmette-Guerin (BCG) and environmental non-tuberculous mycobacteria (NTM). It is a phenotypically heterogeneous syndrome. The clinical features of affected children cover a continuous spectrum from local recurrent NTM infection to disseminated lethal BCG infection. Different types of mutations in four genes (IFNGR1, IFNGR2, IL12B, IL12RB1) have revealed both allelic and non-allelic heterogeneity and result in eight different disorders whose common pathogenic pathway is impaired interferon gamma-mediated immunity (IFNgamma). The severity of the clinical phenotype depends on the genotype. Complete interferon IL-12 p40 and its receptor (beta1-subunit) IL-12 R beta-1 deficiencies and partial IFNgamma-R1 and IFNgamma-R2 deficiencies generally lead to curable infections at various ages. Antibiotics supplemented with IFNgamma, when necessary, are likely to be effective. Complete IFNgamma-R1 and IFNgamma-R2 deficiencies predispose to overwhelming infections in early childhood, which respond poorly to antibiotics and IFNgamma. Rapid discrimination between complete IFNgamma-R1 and IFNgamma-R2 deficiencies and other defects is therefore an important diagnostic step for planning clinical management. The prevalence of idiopathic disseminated BCG-itis in France has been estimated to be at least 0.59 cases per million children vaccinated.

Keywords
predisposition to atypical mycobacterium, Bacillus Calmette-Guerin, IFNGR1, IFNGR2, IL12B, IL12RB1, impaired interferon gamma-mediated immunity (IFNgamma).

Introduction
Bacille Calmette-Guerin (BCG) vaccines and environmental non-tuberculous mycobacteria (NTM) are known to cause severe diseases in immunocompromised children. It is less well known that otherwise healthy children may also be affected (Casanova et al., 1995 and 1996; Frucht et al., 1996; Levin et al., 1995). Unlike children with classic immunodeficiency, who suffer from other clinical diseases caused by various viruses, bacteria, fungi and protozoa, children with severe idiopathic BCG and NTM infections generally do not have other associated symptomatic infections, apart from salmonellosis, which occurs in less than half of them. Clinical diseases caused by Cytomegalovirus (CMV),
Herpes simplex virus (HSV), Listeria monocytogenes and Histoplasma capsulatum have each been found in only one patient (Dorman et al., 1999; Holland et al., 1998, Roesler et al., 1999).

Although these children are not vulnerable to a wide range of infectious agents, the syndrome was thought to be due to impaired immunity, specifically altering host defenses against mycobacteria. Parental consanguinity and familial forms are frequent and the syndrome is often described as Mendelian susceptibility to mycobacterial infection (McKusick, 1998). The clinical syndrome is rare and its inheritance may differ between kindreds. Although autosomal recessive in most cases, autosomal dominant (9) and X-linked recessive inheritance have been reported (Fleisher et al., 1999).

Mutations have been found in four genes (IFNGR1, IFNGR2, IL12B, IL12RB1). Different types of mutations result in eight different disorders, whose common pathogenic mechanism is impaired interferon-gamma (IFNg)-mediated immunity. However, these defects account for only a minority of patients. Interleukin-12 (IL-12), a heterodimeric cytokine, is secreted by phagocytes and dendritic cells, upon infection with mycobacteria. IL-12 is composed of two subunits, p40 and p35, which together form the biologically active p70 heterodimer. IL-12 receptors consist of two chains, IL-12Rb1 and IL-12Rb2, that are selectively expressed on natural killer (NK) and activated T-cells. Binding of IL-12 to these receptors leads to signal transducer and activator of transcription 4 (STAT4) activation, followed by IFNg induction (Gately et al., 1998; Trinchieri et al., 1998). IL-12p40 and IL-12Rb1 deficiencies thus result in impaired IFNg production.

IFNg, a homodimer with pleiotropic effects, is one of the principal macrophage-activating cytokines. IFNg acts through a ubiquitous cell-surface receptor, composed of two chains, IFNgR1, the ligand-binding chain, and IFNgR2, which is required for signal transduction. Upon ligand binding, STAT1, an essential component of the IFNg signaling pathway, is phosphorylated and translocates as a homodimer to the nucleus, where it acts as a transcriptional activator, interacting with the promoter region of IFNg-inducible genes (Bach et al., 1997; Stark et al., 1997). Various types of IFNgR1 and IFNgR2 defects impair cellular responses to IFNg (see below).

These disorders generally manifest in childhood, although they may become apparent during adulthood. The rarity and heterogeneity of the syndrome make accurate diagnosis and treatment difficult for pediatricians, especially because the clinical boundaries are poorly defined, and most patients lack a clear genetic etiology. Herein, we briefly review the known inheritable disorders, emphasizing their molecular pathogenesis, clinical features, diagnosis and management. Reviews of other aspects of the syndrome can be found elsewhere (Dorman et al., 2000; Lammas et al., 2000; Picard et al., 2000).

Prevalence

Cases from various ethnic groups and geographic regions have been reported. The prevalence of the syndrome is difficult to determine as it includes a continuous spectrum from disseminated lethal BCG infection to local recurrent NTM infection. Disseminated forms themselves are heterogeneous, and clinical features correlate with the type of histopathological lesions present (Emile et al., 1997), so that children with lepromatous-like BCG-granulomas generally die of overwhelming infection, whereas children with tuberculoid granulomas have a favorable prognosis. The prevalence of idiopathic disseminated BCG-osis in France has been estimated to be at least 0.59 cases per million children vaccinated (Casanova et al., 1996).

Genetics and immunology

A child with a homozygous recessive deletion in the p40 subunit of IL-12, which leads to complete IL-12p40 deficiency, has been reported (Altare et al., 1998c). Recently, we identified other families with IL-12p40 deficiency, showing that this disorder is not limited to a single kindred (Picard, C. in preparation). Patients with other IL-12 regulation defects and undefined X-recessive genetic defects in trans have been reported (Fucht et al., 1996). Due to a lack of stimulation through IL-12, these patients produce less IFNγ, which may be partially corrected, in a dose-dependent manner, with exogenous recombinant IL-12. This partial correction confirms that the impairment of IFNγ production is not a primary event, but a consequence of an inherited IL-12 deficiency. In other patients, recessive mutations preclude expression of the IL-12Rb1-chain on the surface of NK and T-cells (Altare et al., 1998a; Altare F., submitted). To date, no partial defect of IL-12 or IL-12R has been identified.

Complete IFNγR1 deficiency may be due to recessive mutations, that either preclude surface expression of the receptor ligand-binding chain (Holland et al., 1998; Roesler et al., 1999; Alfare et al., 1998; Jouanguy et al., 1998; Newport et al., 1996; Pierre-Audigier et al.; 1997), or result in normal surface expression of IFNγR1 chains, which do not bind IFNγ (Jouanguy et al., 2000).
One child has been reported to have complete IFNγR2 deficiency due to a homozygous recessive deletion in the coding region of the gene, resulting in a premature stop codon in the extracellular domain (Dorman et al., 1998). Cell-surface expression of IFNγR1 was normal, but no IFNγR2 expression was detected. Other patients have been diagnosed with complete IFNγR2 deficiency, showing that this disorder is not limited to a single kindred (Smith C.I.E. and Holland S.M., personal communication) (Fieschi et al., 2001).

A homozygous recessive mutation, which probably reduces the affinity of IFNγR1 for its ligand, has been identified in two siblings (Jouanguy et al., 2000). Cell-surface expression of the receptor ligand-binding chain was normal. Another patient had a homozygous recessive mutation in the IFNγR2 chain, with impaired responses to IFNγ and normal surface expression (Döffinger et al., 2000). Both recessive defects led to partial, as opposed to complete, IFNγR2 deficiency.

An autosomal dominant form of partial IFNγR1 deficiency was found in eighteen patients from twelve unrelated kindreds (Jouanguy et al., 1999). The mutant allele encodes a truncated intracellular receptor that binds IFNγ with normal affinity but cannot transduce IFNγ-triggered signals and accumulates on the cell surface. The combination of normal IFNγ binding, impaired signaling and the accumulation of the receptor on the cell surface (five to ten times the normal number of receptors) accounts for the dominant negative effect of the truncated mutant IFNγR1 molecules. Most IFNγR1 molecules are non-functional, but the few wild-type dimers that do form cause the defect to be partial rather than complete.

Histological and clinical features
A multicenter survey is underway to define precisely the clinical and histological phenotypes of patients with the syndrome and each of the underlying genetic defects. For all patients known to date, mycobacterial infection has been the principal clinical presentation and none have clinical atopy or serological evidence of sensitization to common aero-allergens or signs of autoimmunity (Wood, in preparation and (Döffinger et al., 1999).

Generally, patients with IL-12 and IL-12 Receptor deficiencies have mild symptoms, with delayed but good granuloma formation in response to BCG vaccination (Altare et al., 1998) and impaired granuloma formation following NTM infection. The child with complete IL-12p40 deficiency presented with curable BCG- and Salmonella enteritidis- infections (Altare et al., 1998) and the patients with IL-12 regulation deficiency had Mycobacterium avium infections (Frucht et al., 1996). The milder clinical phenotype in comparison to children with complete IFNγR deficiencies is probably due to residual, albeit low IL-12-independent IFNγ-mediated immunity.

The patients with complete IL-12RB1 deficiencies presented with curable BCG infections upon vaccination and NTM infections after the age of three years, with these infections not occurring until adulthood in two. In one patient, the infection was fatal. Surprisingly, the sister of one of the patients with BCG infection was resistant to three inoculations of BCG, but developed abdominal tuberculosis at the age of 18 years. The two siblings thus had the same genotype, but different clinical phenotypes (Altare F. et al., submitted). That observation demonstrates phenotypic heterogeneity for a given genotype in IL-12R1 deficiency. Moreover, it raises the question as to whether other children with severe tuberculosis may have IL-12RB1 deficiencies.

The clinical phenotype of patients with partial IFNγR deficiency is generally mild like that in IL-12R deficiency. One patient with partial recessive IFNγR1 deficiency presented with clinical BCG and Salmonella enteritidis infections and the other patient, who had not been vaccinated, had symptomatic tuberculosis. In all tissue biopsies, mature granulomas were seen. The patient with partial recessive IFNγR2 deficiency had a history of BCG and Mycobacterium abscessus infections. In the dominant forms of partial IFNγR deficiency, the clinical phenotype is mild even though the course of infection appears to be somewhat more severe than in partial recessive IFNγR deficiency. Patients with complete IFNγR1- or IFNγR2 deficiency suffer from a severe form of the syndrome, with BCG infection after immunization and early onset NTM infection (often before three years of age). Lethal infection due to Mycobacterium smegmatis, one of the least virulent mycobacteria and which had never been previously identified as a cause of disseminated clinical disease, has occurred once. Other rapidly growing mycobacterial species, such as M. fortuitum, but also M. avium, Salmonella and Listeria monocytogenes infections have been reported. Severe diseases caused by viruses, such as CMV and HSV have each been diagnosed in one child (5, 7, 26, 28). No other opportunistic infections were observed and common childhood infections followed a normal course. Lepromatous-like lesions, particularly in response to BCG vaccination, were observed and are suggestive of complete IFNγR1 or IFNγR2 deficiency.
There is a correlation among genotype and cellular and clinical phenotypes for patients with IFNgR deficiencies. Thus IFNγ-mediated cell activation is a genetically controlled continuous quantitative trait that determines the outcome of mycobacterial infection in humans (Dupuis et al., 2000). In contrast, the clinical phenotype of patients with IL-12 or IL-12R gene defect is heterogeneous among and within families.

Diagnosis

Rapid diagnosis of complete IFNγR deficiency is essential for the planning of clinical management and can be made by determining serum IFNγ levels by ELISA (Fieschi, 2001). High IFNγ levels suggest complete IFNγR deficiency, whereas low or undetectable levels indicate IL-12, IL-12R, partial IFNγR or undetermined defects (Holland et al., 1998). More specialized assays are necessary to determine the exact molecular etiology of the disease in each patient. IL-12p40 deficiency can be diagnosed by ELISA, with low levels of IL-12p40, IL-12p70 and IFNγ secretion by stimulated peripheral blood mononuclear cells (PBMC). IL-12Rβ1 deficiency results in low levels of IFNγ production and FACS analysis cannot detect the IL-12Rβ1 chain on activated T-cells. Gene sequencing and, depending on the mutation, in vitro gene transfer are needed to confirm the diagnosis. Several functional studies have assessed the cellular responses to recombinant IFNγ. ELISA can be used to quantify tumour necrosis factor γ (TNFγ) production by blood cells in response to lipopolysaccharide (LPS) alone, and LPS plus IFNγ (Levin et al., 1995, Jouanguy et al., 1996). STAT1 nuclear translocation can be determined by electrophoretic mobility shift assay, using cultured Epstein-Barr virus (EBV) transformed B-cells (Jouanguy et al., 1997). Alternatively, it is possible to detect intracellular phosphorylated STAT1 by flow cytometry (Fleisher et al., 1999). HLA-DR surface induction can be detected in SV40-transformed fibroblasts by flow cytometry with specific antibodies (Altare et al., 1998b). Cells from patients with complete IFNγR deficiency do not respond to IFNγ, even at high concentrations (105 IU/ml), whereas cells from patients with partial deficiencies respond to high but not to low concentrations of IFNγ. In addition, over expression of the IFNγR1 chain in the autosomal dominant form facilitates rapid diagnosis of this form of partial IFNγR1 deficiency by FACS analysis. Here too, gene sequencing and, depending on the mutation, in vitro gene transfer provide the molecular diagnosis of the patients (Altare et al., 1998a; de Jong et al., 1998).

Treatment and prognosis

For all patients, appropriate antibiotic therapy, based on the susceptibilities of the mycobacterial species identified, is crucial. However, for initial empirical therapy a history of BCG vaccination is important and unvaccinated children should be considered as infected by NTM, though tuberculosis should be considered in some cases. Antimycobacterial therapy may have to be continued for extended periods and supplementary measures, such as the drainage of pus, attention to nutrition and growth, are important. Occasionally, surgical resection of refractory infectious sites, such as abdominal lymphnodes, may be required. BCG immunization is contraindicated and it is prudent to avoid other live vaccines. Patients with IL-12, IL-12R and partial IFNγR defects usually respond well to antibiotic treatment and in those who do not respond well, additional IFNγ therapy has been shown to be effective. Experience shows that this option should be considered individually for each patient. An initial dose of 30 to 50 µg/m2 given subcutaneously three times a week should be adapted according to clinical and in vitro responses. Doses as high as 500µg/m2 may be necessary (Lammas et al., 2000).

The molecular diagnosis of complete IFNγR1 and IFNγR2 deficiency has important therapeutic implications, as mycobacterial disease in patients with such deficiencies is overwhelming and refractory to antibiotic treatment. Bone-marrow transplantation probably remains the treatment of choice, because IFNγ is ineffective in the absence of specific functional receptors. However, transplantation has proved difficult in several patients (unpublished observations). Gene therapy should be developed in the future for the treatment of complete IFNγR deficiencies, but further research is required to make this possibility a reality.

Conclusion

Mendelian susceptibility to mycobacterial infection is a rare and heterogeneous syndrome of childhood that is probably underdiagnosed. The clinical features of affected children range from disseminated lethal BCG infection to local and recurrent NTM infection. Various mutations in IFNGR1, IFNGR2, IL12B and IL12RB1 define eight genetically different disorders. These disorders are immunologically related, as impaired IFNγ-mediated immunity is the common pathogenic mechanism underlying mycobacterial infection in all patients. The severity of the clinical phenotype depends on the genotype. Complete IL-12p40 and IL-12Rβ1 deficiencies and partial
IFNγR1 and IFNγR2 deficiencies generally predispose the patient to curable infections at various ages. Antibiotics, supplemented with IFNγ when necessary, are likely to be effective and bone-marrow transplantation is therefore not indicated. Complete IFNγR1 and IFNγR2 deficiencies predispose the patient to overwhelming infection in early childhood. The response to antibiotics is poor and IFNγ treatment ineffective, which probably makes bone-marrow transplantation the treatment of choice. There is a genotype-phenotype correlation for IFNγR defects unlike IL-12 and IL-12R defects. Rapid discrimination between complete IFNγR deficiency and other defects by ELISA determination of serum IFNγ levels is an important diagnostic step for planning clinical management. However, diagnosis of the molecular defect remains difficult, as the syndrome is heterogeneous and few standardized assays are available. Tests to detect IFNγ and IL-12 cytokines and their receptors, followed by functional assays, gene sequencing and in vitro gene transfer provide a definite diagnosis in only a minority of cases. The treatment of other patients with unknown genetic defects remains empirical. Future research will focus on the search for other underlying genetic defects to provide a rational basis for the management of patients with mycobacterial disease.

References


Jouanguy E, Dupuis S, Pallier A, et al. 2000. In a novel form of IFN-gamma receptor 1 deficiency,


http://www.orpha.net/data/patho/GB/uk-bcgile.pdf
cell surface receptors fail to bind IFN-gamma. *J Clin Invest* 105: 1429-36


