Infections Due to Various Atypical Mycobacteria in a Norwegian Multiplex Family with Dominant Interferon-γ Receptor Deficiency

Heidi Glosli, 1 Asbjørg Stray-Pedersen, 1 Anne C. Brun, 1 Lena W. Holtmon, 1 Tone Tonjum, 1, 2 Ariadne Chapgier, 3 Jean L. Casanova, 5, 6 and Tore G. Abrahamsen 1, 3

1 Department of Pediatrics, and 2 Institute of Microbiology, Rikshospitalet University Hospital, and 3 Medical Faculty, University of Oslo, Oslo, and 4 Department of Pediatrics, Vestfold Hospital, Tønsberg, Norway; and 5 Laboratoire de Génétique Humaine des Maladies Infectieuses, INSERM U550, Faculté de Médecine Necker-Enfants Malades, and 6 Unité d’Immunologie et d’Hématologie Pédiatriques, Hôpital Necker-Enfants Malades, Paris, France

Background. Atypical mycobacteria can cause systemic infections in patients with certain types of immunodeficiency.

Methods. Clinical samples were decontaminated and cultured to assess the presence of mycobacterial species. Gene sequencing was performed to reveal interferon-γ receptor 1 (IFN-γR1) deficiency.

Results. The index patient received a diagnosis of dominant IFN-γR1 deficiency during treatment for a serious infection due to atypical mycobacteria. She belongs to a Norwegian multiplex family comprising 3 generations and 5 patients with dominant IFN-γR1 deficiency. Four of these patients have been treated with tuberculostatics because of extensive infection due to atypical mycobacteria, such as Mycobacterium avium-intracellulare, Mycobacterium scrofulaceum, Mycobacterium bovis (bacille Calmette-Guérin), Mycobacterium bohemicum, and Mycobacterium gordonae. Two of the patients have also received subcutaneous injections of IFN-γ. One family member with the deficiency has not received treatment and is still healthy at 13 years of age.

Conclusions. Serious infection due to atypical mycobacteria should initiate a search for primary immunodeficiencies, particularly IFN-γR1 deficiency. Treatment with IFN-γ should be started when serious infection due to atypical mycobacteria is verified and dominant partial IFN-γR1 deficiency is suspected.
agents; the 2 youngest patients have also received injections of IFN-γ 3 times weekly. Our index patient presented with a prolonged history, disseminated infection due to the atypical mycobacterium Mycobacterium avium-intracellulare, and a family history of similar infections.

**METHODS**

Clinical samples were investigated for the presence of mycobacteria by the standard methods at the time. Recently, the samples have been cultivated at 37°C in the Bactec MGIT 960 system (Becton-Dickinson). Isolates that were nontuberculous mycobacteria were identified either by GenProbe hybridization (for *M. avium-intracellulare*) or 16S rDNA PCR sequence analysis (for all other nontuberculous mycobacteria).

The sequencing of IFNGRI was performed as described elsewhere [5]. The findings were confirmed by gel electrophoresis and direct sequencing of genomic PCR products obtained for each exon and flanking intronic region.

Biochemical activation of the IFN-γ pathway was evaluated by means of electrophoretic mobility shift assay, as described elsewhere [7]. Nuclear protein extracts from the patient and a positive control sample (Epstein-Barr virus type B cells) were incubated with a radiolabeled γ-activated sequence DNA probe after several doses of IFN-γ.

The activation of IFN-γ at the cellular level was performed as previously described [8]. In short, a whole blood sample was activated with bacille Calmette-Guérin (BCG) plus either IFN-γ or IL-12, for investigation of IL-12p40 and IL-12p70 or of IFN-γ production, respectively.

**RESULTS**

**General findings.** Sequencing of chromosome 6 revealed a deletion of 4 nucleotides in exon 6 of IFNGRI, designated 818del4 (OMIM 107470.0006), implying a dominant, partial IFN-γR1 deficiency in 5 members of the family. The biochemical activation of IFN-γ studied by electrophoretic mobility shift assay showed a partial deficiency in IFN-γ response in these patients, compared with control subjects (data not shown), as described elsewhere for 818del4/WT cells from patients [5].

At the cellular level, all of our heterozygous patients showed a partial defect in IL-12p40 and a complete defect in production of IL-12p70 to BCG plus IFN-γ, whereas they had normal responses to BCG alone or to BCG plus IL-12 for the production of IFN-γ (data not shown), as described elsewhere for 818del4/WT whole blood cells [8].

**Patient 1.** A 7-year-old girl (born in 1995) (figure 1 and table 1) was admitted to the hospital with an infiltrate in the upper part of the left lung 4 months after having a possible Schönlein-Henoch purpura. Treatment with oral penicillin G and aerosolized salbutamol was given for 10 days. However, 4 weeks after discontinuation of the treatment, a new chest radiograph revealed a confluent infiltrate in the upper part of the left lung. Supplemental pulmonary CT exhibited a single abscess with a diameter of 2 cm and several microabscesses in the same lung, whereas an abdominal CT showed a 3 × 4 cm—abscess in the lateral part of the right liver lobe. Treatment with cefotaxime and, subsequently, metronidazole was started. A tuberculin skin test (Pirquet’s test) with regular tuberculin for *M. tuberculosis* yielded positive results (diameter at 2 different test sites, 2 mm and 3 mm). The girl was transferred to the reference hospital for drainage of the liver abscess and further evaluation. She had fever and was pale, with enlarged lymph nodes, especially in the neck region and both sides of the groin. The liver was enlarged, and ultrasound analysis revealed an additional abscess. She had a limping gait.

Pus from one of the liver abscess contained *M. avium-intracellulare*. Scintigraphy revealed massive bone involvement. Treatment was started with oral rifabutin, clarithromycin, and ethambutol and intravenous amikacin. The patient’s clinical condition improved slightly. However, the mycobacteria isolated were resistant to amikacin, rifabutin, clarithromycin, and ciprofloxacin, whereas they were susceptible to ethambutol and ethambutol in combination with clarithromycin or ciprofloxacin. Therefore, only the treatment with ethambutol and clarithromycin was continued. The patient developed an abscess with cheesy content in labia majora. Microbiological culture revealed the presence of *Enterococcus faecium* that was susceptible only to vancomycin and linezolid. Linezolid was added to her treatment regimen, and she received local treatment with drainage. Despite these efforts, the infection developed into pelvic osteomyelitis, which healed after 12 months. After 4 weeks of treatment, the *E. faecium* isolate was susceptible to penicillin, ampicillin, and amoxicillin but had become resistant to linezolid. Linezolid was therefore replaced with ciprofloxacin.

Because of the family history of systemic atypical mycobact-
teria infections, treatment with IFN-γ was started as soon as immunodeficiency was suspected, ~6 months after the first sign of disease. To date, the patient is still receiving treatment with ethambutol, clarithromycin, ciprofloxacin, and IFN-γ. Treatment with IFN-γ is thought to overcome the partial deficiency of IFN-γR1 in these patients [5].

Patient 2. A man born in 1953 (figure 1 and table 1) started to limp and complained of pain when running when he was ~6 years of age. Osteomyelitis in his right heel was diagnosed when he was 8 years of age. At the time of diagnosis, the patient had a negative tuberculin skin test (Pirquet’s test) result. Biopsy of the right heel revealed granulomatous infection, and culture of the biopsy specimen yielded growth of Mycobacterium scrofulaceum. The patient was treated with curettage of the right heel, in addition to streptomycin, paraaminosalicylate, and isoniazid for 8 months.

At 14 years of age, the patient received vaccination with BCG vaccine. Two months later, he was admitted to the hospital because of cachexia, anemia, and multiple osteolytic lesions in the skeleton. Examination of material from the lesions revealed growth of atypical mycobacteria (BCG subtype). The patient received surgical treatment (curettage and bone transplantation) of the left humeral metaphysis, calvarium, and right tibial metaphysis, as well as antibiotic treatment with streptomycin, paraaminosalicylate, and isoniazid for 12 months. Pirquet’s test 5 months after vaccination yielded a positive reaction (diameter, 5 mm), but after 12 months, the reaction was vesicular, which represents a significantly positive result.

Later, in 1979, the patient again had symptoms that were similar to his previous symptoms, including cachexia, fatigue, neck pain, tender spots in the skull, and a highly elevated erythrocyte sedimentation rate [9]. Radiological examination revealed osteolytic lesions in the skull. On scintigraphy, multiple pathological enhancements in cranium, upper and lower extremities, columna, costae, and pelvis were found. A liver biopsy revealed granulomas, and culture of material obtained from the skull by biopsy revealed acid-fast rods. By culture of bone tissue, both M. avium-intracellulare complex and M. scrofulaceum were isolated [9]. The patient was treated with rimactan, isoniazid, ethambutol, trevintix, and pyrazinamide for 6 years. After 4 years of treatment, it was still possible to obtain M. avium-intracellulare complex by culture of a urine specimen from the patient, and scintigraphy revealed unchanged signal enhancement in the sixth costae and the right part of the pelvis. The antimycobacterial treatment was stopped in 1986, and there has been no evidence of relapse to date. In 2000, the patient received a diagnosis of chronic obstructive pulmonary disease.

Patient 3. A man born in 1975 (figure 1 and table 1) had recurrent upper airway infections at 8 years of age, and enlarged lymph nodes in the neck region were reported. At 13 years of age, the patient spontaneously began having a positive vesicular reaction to a tuberculin skin test (Pirquet’s test). The lymph nodes continued to grow, and an extirpation was performed when he was 14 years of age. Granulomatous infection was found by histological examination. In addition, culture of biopsy material from the lymph node revealed the presence of Mycobacterium gordonae. Two years later, the patient’s lymphadenitis worsened, and he complained of fatigue. At this time, he received isoniazid, rifampicin, and ethambutol for 6–12 months. Because of his father’s history of systemic infection after BCG vaccination, he was not vaccinated with BCG vaccine.

Patient 4. A girl born in 1999 (figure 1 and table 1) received a diagnosis of systemic infection due to atypical mycobacteria after the detection of autosomal dominant IFN-γR1 deficiency. At a routine follow-up visit in 2004 because of her known gene defect, an abscess was discovered in the left lumbosacral region. MRI showed involvement of soft tissue and skeleton. During biopsy, granulation tissue with acid-fast rods was found. Treatment was started with rifabutin, clarithromycin, ethambutol, and amikacin, in addition to subcutaneous IFN-γ injections 3 times weekly. Culture of the biopsy material exhibited growth of Mycobacterium bohemicum. The treatment with amikacin was ended after 6 weeks, whereas therapy with the other antimycobacterial agents and IFN-γ was continued. The lumbosacral abscess disappeared slowly, and the patient has otherwise been healthy.

Patient 5. In 2003, a boy born in 1993 (figure 1 and table 1) was discovered to have the familial deletion of IFNGRI, 818del4; other members of his family had this deletion as well. The patient has, thus far, been checked regularly at the hospital,

Table 1. Characteristics of the patients in a study of IFN-γ receptor 1 deficiency and infection due to atypical mycobacteria.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Age at diagnosis of mycobacterial infection, years</td>
<td>7</td>
<td>6</td>
<td>13</td>
<td>5</td>
<td>...</td>
</tr>
<tr>
<td>Strain of Mycobacterium</td>
<td>M. avium-intracellulare</td>
<td>M. scrofulaceum, bacille Calmette-Guérin</td>
<td>M. gordonae</td>
<td>M. bohemicum</td>
<td>None</td>
</tr>
<tr>
<td>Affected tissues</td>
<td>Liver, lung, lymph nodes, skeleton, and soft tissue</td>
<td>Lung and skeleton</td>
<td>Lymph nodes</td>
<td>Skeleton and soft tissue</td>
<td>None</td>
</tr>
<tr>
<td>Subcutaneous IFN-γ treatment</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

IFN-γR1 Deficiency and Atypical Mycobacteria • CID 2008;46 (1 February) • e25
and there has never been signs of infection due to atypical mycobacteria.

DISCUSSION

In this family, consisting of 1 ancestor and 5 descendants in 2 generations (figure 1), the ancestor and 4 of his descendants were described as having an autosomal dominant defect of the gene encoding IFN-\(\gamma\)R1, giving rise to a selective immunodeficiency. The mutation described in this family is the most common defect described in IFNGR1 [10]. It consists of a deletion of 4 nucleotides flanking position 818 in the gene encoding IFNGR1, located on chromosome 6q23-q24, designated 818del4 [11]. This deletion is just 3′ of the segment encoding the transmembrane region and results in a stop codon at amino acid position 276, leaving the cytoplasmic region only 5 amino acids long, compared with 187 amino acids in the wild type. This implies that the encoded IFN-\(\gamma\)R1 variant is expressed on the cell surface but lacks most of the cytoplasmic domain [5]. Because the wild-type cytoplasmic domain contains the binding sites for JAK1 and STAT1, as well as a recycling/internalization motif, the mutant protein is impaired in cellular signaling and recycling, while retaining normal binding affinity for IFN-\(\gamma\) [5]. As a result, the mutant proteins accumulate at the cell surface and act as antagonists for wild-type IFN-\(\gamma\)R1 proteins, thereby exerting a dominant negative effect [12]. The patients with dominant IFN-\(\gamma\)R1 deficiency are only partially deficient to IFN-\(\gamma\), probably because of homodimerization of the minority of cell-surface wild-type receptors [5].

An interesting clinical feature of persons with this dominant negative mutation is that they frequently develop multifocal osteomyelitis due to mycobacteria [5, 10, 13], which is rarely seen in other persons. This implies the necessity to search for deletions in IFNGR1 when a multifocal osteomyelitis due to mycobacteria is diagnosed. In this study, as well as in others, the clinical course of the same genetic defect is quite variable within the same family [14, 15]. Factors that modify the outcome are unknown but may include the strain, antigenicity, and virulence of the mycobacteria, the size of the infectious inoculum, and the general health condition and the presence of compensating host defenses and receptor profile of the affected individual. Other genes may be involved in modulating the degree of susceptibility to infection. This could explain the occurrence of this specific IFN-\(\gamma\)R1 mutation in asymptomatic, exposed persons, including 1 family member in our study [5, 10, 13, 14, 16–20]. According to Dorman et al. [10], the prevalence of mycobacterial infection was 95% among patients with IFN-\(\gamma\)R1 deficiency, compared with 80% (4 of 5 patients) in our study. The first environmental mycobacterial infection in our study was diagnosed at a mean age of \(\sim\)8 years (table 1), compared with 13.4 years in the study by Dorman et al. [10].

Dorman and colleagues described 2 tissues affected, whereas we have found 2–5 (table 1). Even if there seems to be some differences between our study and the study by Dorman and colleagues, these cannot be verified, because our family consists of only 5 affected persons.

Mycobacteria, especially environmental strains with low virulence, are the main threat to patients with IFN-\(\gamma\)R1 deficiency. It is very important that these patients undergo regular follow-up consultations. Antimyobacterial treatment should be started promptly when signs of infection appear. The patients should not be vaccinated with BCG vaccine, because 4 of 5 vaccinees in the study by Dorman et al. [10] contracted a systemic BCG infection, although the vaccination seemed to postpone infections due to environmental mycobacteria. Increased awareness of the clinical features of dominant partial IFN-\(\gamma\)R1 deficiency should facilitate accurate diagnosis and treatment. The rationale for treating the patients with IFN-\(\gamma\) is that an excessive supply of IFN-\(\gamma\) overcomes the partial lack of functional IFN-\(\gamma\)R1 in the cells [10].

In patients with recessive IFN-\(\gamma\) deficiency, on the other hand, treatment with IFN-\(\gamma\) has no effect, because of either surface expression of a nonfunctional molecule or lack of expression of the protein on the cell surface [11]. A thorough characterization of patients with IFN-\(\gamma\) deficiency is important for planning further treatment. In this family, the male individuals did not receive treatment with IFN-\(\gamma\), because the underlying deficiency was not known at the time. On the other hand, the 2 female individuals have received subcutaneous IFN-\(\gamma\) therapy for \(\sim\)4 years. They are now doing very well clinically and exhibit no signs of mycobacterial infection. It is difficult to know the optimal duration of IFN-\(\gamma\) treatment. The male individuals in our study have not received therapy and have not shown signs of mycobacterial infection for years. Because persons with IFN-\(\gamma\)R1 deficiency are quite rare, the study of the role of IFN-\(\gamma\) in a randomized way is impossible. Physicians caring for such patients must rely on reasoning and, perhaps, animal data. Ehlers and Richter [21] have described the role of IFN-\(\gamma\) in the murine defense against mycobacteria. They concluded that IFN-\(\gamma\) is crucial in the defense against some but not all opportunistic infectious agents [21]. It would be interesting to study the role of excessive IFN-\(\gamma\), which has theoretical justification to be administered, in animal models with appropriate immunodeficiencies. Treatment with subcutaneous IFN-\(\gamma\) should be started when serious infection with atypical mycobacteria is verified and dominant partial IFN-\(\gamma\)R1 deficiency is suspected or verified.

The taxonomy of mycobacterial species has evolved significantly during the past 30 years. After the introduction of genetic methods, much more detailed resolution at the genetic level has been possible, despite similar phenotypic traits. Nontuberculous mycobacterial lymphadenitis, traditionally caused
by *M. scrofulaceum*, has been shown to be most often due to members of the *M. avium-intracellulare* complex [22]. *M. bohemicum*, a slow-growing scotochromogenic mycobacterium with a phenotype quite similar to that of *M. scrofulaceum*, was described for the first time in 1998 [23]. Since this discovery, this species has been described as a cause of granulomatous lymphadenitis in immunocompetent patients [24, 25] and of infections in animal models [21].

When multifocal osteomyelitis or a serious infection with nontuberculous mycobacteria is diagnosed in otherwise healthy persons, it is important to search for deficiencies in the innate immune system, including the IFN-γ signaling pathway. By discovering such immunodeficiencies early, it is possible to provide the patient with substitute treatment and, thereby, prohibit morbidity. It is very important to avoid BCG vaccination for these patients.

**Acknowledgments**

**Potential conflicts of interest.** All authors: no conflicts.

**References**