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CASE REPORT

Disseminated *Mycobacterium scrofulaceum* infection in a child with interferon-γ receptor 1 deficiency

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Summary Disseminated disease caused by non-tuberculous, environmental mycobacteria (EM) reflects impaired host immunity. Disseminated disease caused by *Mycobacterium scrofulaceum* has primarily been reported in patients with AIDS. Moreover, observing *M. scrofulaceum* as the agent of localized disease in childhood has become increasingly rare. We report the first case of disseminated disease caused by *M. scrofulaceum* in a child with inherited interferon-γ receptor 1 (IFN-γR1) complete deficiency. As in this case, mycobacterial bone infections in IFN-γR1 deficiency can sometimes mimic the clinical picture of chronic recurrent multifocal osteomyelitis.

Introduction

Mycobacterium scrofulaceum is one of the best known mycobacterial species, and was described almost 50 years ago.[1]

Traditional microbiology describes *M. scrofulaceum* as a slow growing scotochromogenic species that is characterized by negativity of almost all the most frequently performed biochemical tests, but showing consistently positive catalase assays. The most popular phylogenetic school, which is based on the nucleotide sequence of the gene encoding the 16S rDNA, places *M. scrofulaceum* in an isolated evolutionary branch not closely related to any other species. This classification likely reflects the unique sequence of *M. scrofulaceum* in helix 18, which is characterized by the presence of a

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9-nucleotide insertion that differentiates *M. scrofulaceum* from slow growers (presenting a 12-nucleotide insertion) and from rapid growers (lacking any insertion).[2]

The name *M. scrofulaceum* derives from 'scrofula', an infection of the cervical lymph nodes. It refers to the high frequency with which this species is found in such lesions in pre-school children.[3] Recently, however, isolating *M. scrofulaceum* in childhood lymphadenitis has become increasingly rare, while the incidence of other non-tuberculous, environmental mycobacteria (EM), especially *Mycobacterium avium* and several newly identified species, has increased.[2,3] Far less commonly observed clinical manifestations of *M. scrofulaceum* infection in adults include pulmonary disease,[4] osteomyelitis,[5] hepatitis,[6] and disseminated disease,[7,8] primarily in AIDS patients,[9] while intrathoracic and, more rarely, disseminated disease are reported in children.[10,11]

To the best of our knowledge, no cases of disseminated disease caused by *M. scrofulaceum* have been reported in any patients at all with primary immunodeficiency, except in a patient with a dominant interferon-γ (IFN-γ) receptor deficiency.[12]

Case report

An Italian boy, aged 2 years and 10 months, was admitted to a local hospital because of fever and pain in his right leg, right foot, and left hand. His parents were born in the same small town, yet consanguinity was not documented. The child had not been inoculated with bacille Calmette—Guérin (BCG). On admission, the patient appeared to be in a severe clinical condition: his weight and height were under the 5th percentile, and laboratory tests revealed severe hypochromic anemia, thrombocytosis, leukocytosis, and elevated inflammatory indices. Morphological bone marrow examination ruled out leukemia, and radiological studies showed multiple disseminated osteolytic lesions. Bone biopsy showed paratrabecular fibrosis, partial lamellar resorption with no signs of necrosis. Unfortunately, bone biopsy culture was not performed. Intravenous antibiotics (ceftriaxone, amikacin, teicoplanin for 18 days) and non-steroidal anti-inflammatory drugs led to a transient improvement in pain, and the patient was discharged with a diagnosis of suspected chronic recurrent multifocal osteomyelitis.

Three months later, the child was referred to our institute because of persistent abdominal pain, limb pain, and gait problems. He was in a poor general condition with persistent fever. Laboratory tests showed severe anemia (hemoglobin 6.3 g/dl), leukocytosis (52.92 $\times$ 10^9/l), thrombocytosis (platelets 1065 $\times$ 10^9/l), and elevated inflammatory markers. The results of immunological testing (serum levels of immunoglobulins, peripheral B and T lymphocytes, CD4/CD8 subset, complement) were normal. Chronic granulomatous disease was ruled out. Serological tests for *Borrelia burgdorferi*, *Bartonella henselae*, *Yersinia enterocolitica*, and *Yersinia pseudotuberculosis*, toxoplasmosis, cytomegalovirus, and HIV infection were negative. A Mantoux test was 5 mm after 48 hours and negative after 72 hours. Bone marrow biopsy was negative.

Radiological study of the hands and legs showed an alteration of the bone structure (Figure 1). Magnetic resonance imaging (MRI) of the legs, feet, and hands showed multiple

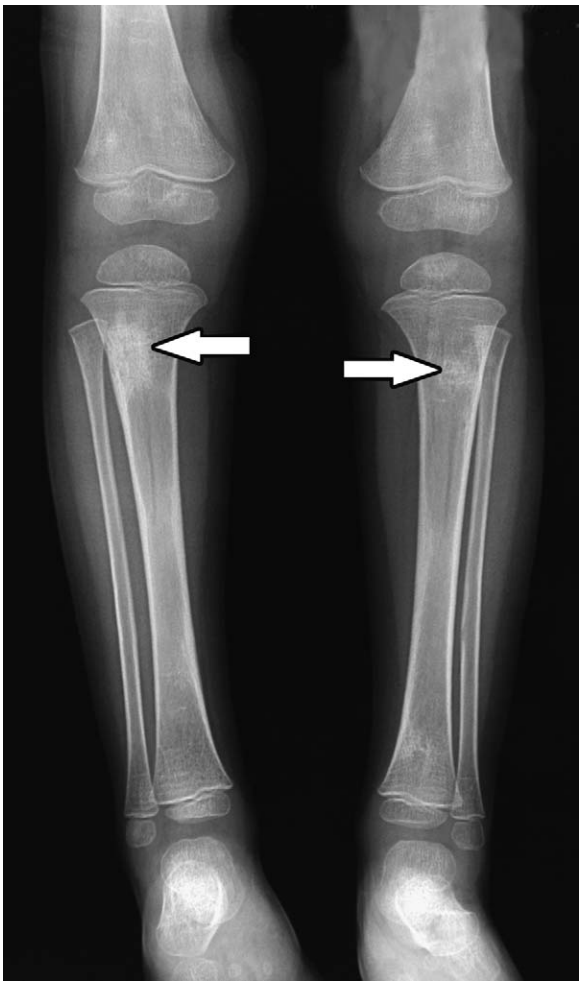

Figure 1 X-ray of the legs: mixed bilateral alteration of the bone structure of the tibial diaphysis (arrows).

focal lesions of the medullary region with metaphyseal, epiphyseal, and diaphyseal involvement of the long bones (Figure 2). A computed tomography (CT) scan of the abdomen revealed a hypodense lesion in the left lobe of the liver and enlarged mesenteric and peripancreatic lymph nodes. A mesenteric lymph node biopsy was performed because of suspected histiocytosis, and histological examination revealed 'granulomatous necrotizing lymphadenitis'. A prevalent neutrophilic infiltration associated with histiomacrophagic reaction was observed, while no epithelioid cells or giant Langhans multinucleated cells were found (Figure 3). Acid-fast rods were visible in the biopsy specimens. Culturing the lymph node yielded acid-fast bacilli. Anti-tuberculous therapy with isoniazid, rifampin, ethambutol, pyrazinamide, and streptomycin was started. As soon as rifabutin- and clarithromycin-sensitive *M. scrofulaceum* was identified, treatment with rifampin, rifabutin, and clarithromycin was started. The child improved and MRI showed no progression of bone lesions. This therapy was continued for ten months.

Three months after therapy was discontinued, the patient presented recurrence of high fever, severe pain, and swelling of the hands and feet, and was re-admitted to our unit. The child was unable to walk, and enlarged lymph nodes at the popliteal and epitrochlear sites were evident. An MRI scan of the legs revealed diffuse alterations of the signal, involving

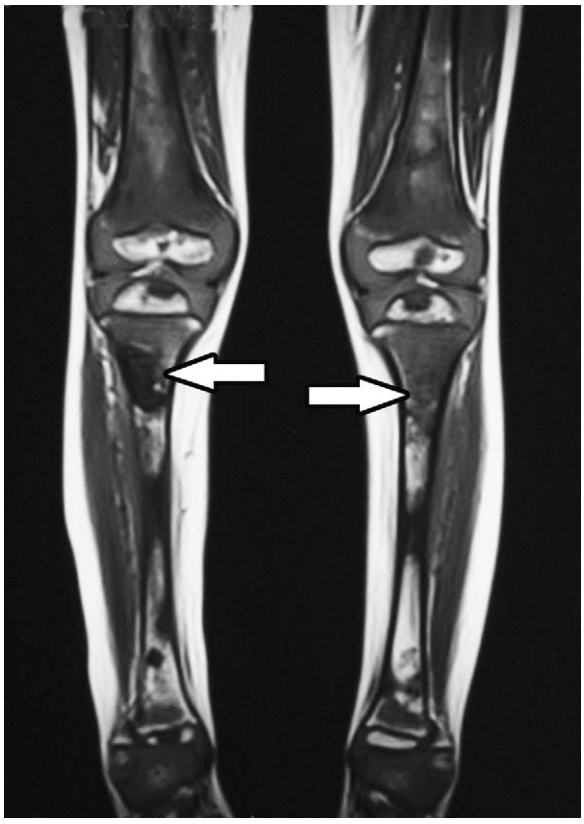

Figure 2 MRI T1-weighted sequences: multiple focal lesions of the medullar region involving the long bones of the legs (arrows).

the medullary region of the long bones of the lower limbs and of the pelvis. A biopsy of the epitrochlear and popliteal lymph nodes was performed and histological examination revealed numerous acid-fast bacilli in both samples. Culture was positive for *M. scrofulaceum*. Disseminated *M. scrofulaceum* infection was diagnosed on the basis of the recurrence of the same mycobacterial infection (positive culture from various lymph node biopsies), the child's severe clinical condition (multiple bone lesions), and the good response to specific antibiotic therapy.

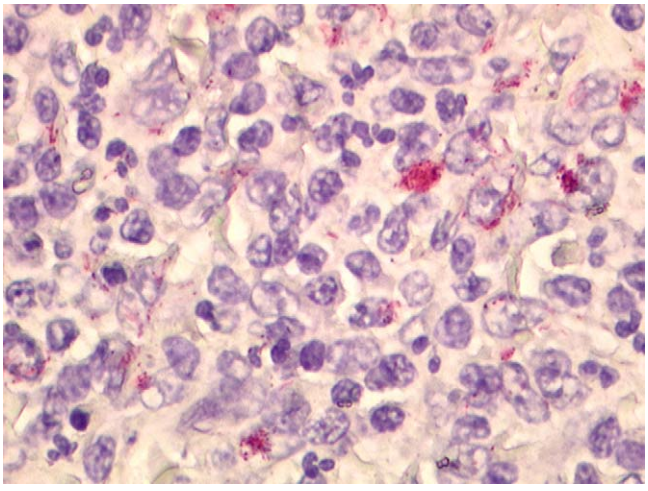

Figure 3 Mesenteric lymph node biopsy: altered structure with neutrophilic infiltration associated with histiomacrophagic reaction. The Ziehl—Neelsen coloration highlights the presence of numerous acid-alcohol resistant bacilli in the cytoplasm of the histiocytes (100 $\times$).

Diagnostic evaluation of the IFN-γ/interleukin-12 (IL-12) axis

We then suspected a defect of innate immunity, thus IFN-γ/IL-12 axis defects were investigated. An ex vivo whole blood assay showed a complete defect of response to BCG and BCG + IFN-γ stimulation in terms of IL-12p40 and IL-12p70 production and only a mild defect of response to BCG and BCG + IL-12 in terms of IFN-γ production, as can be observed in patients with complete IFN-γ receptor deficiencies (data not shown). Genes of the IFN-γ pathway were then sequenced and homozygous deletion of a thymidine was found in exon 4 at the genomic level and at position 523 at the cDNA level in the IFN-γ receptor 1 (IFN-γR1) gene (data not shown). The 523delT mutation leads to a premature stop codon at position 526—528. It is associated with a complete defect of IFN-γR1 expression and function and is inherited in an autosomal recessive mode.[13] Antimycobacterial therapy was resumed and the patient was discharged on therapy with streptomycin, clarithromycin, rifabutin, and ethambutol.

Subsequently, the patient was examined every month and his general clinical condition slowly improved. A radiographic study of the legs showed an improvement in the previous diffuse lesions. Six months after discharge, we treated the child with acyclovir due to a contact with an individual affected by chickenpox. The patient did not develop the disease, but anti-varicella zoster virus antibodies were detected in his serum. Currently, the child is in a good, stable clinical condition and is continuing home therapy with clarithromycin, rifabutin, and moxifloxacin in the absence of any signs of EM infection.

Microbiology

Identification was carried out on two different strains that were isolated 14 months apart. The first strain was isolated from a mesenteric lymph node and the second one from a popliteal lymph node. In both cases, a commercial line probe assay (INNO LiPA, Innogenetics, Belgium)[14] unquestionably assigned the organism to the *M. scrofulaceum* species. Very recently, however, a study pointed out that by using commercial DNA probes, the hybridization results of a newly described species, i.e., *Mycobacterium parascrofulaceum*,[15] are undistinguishable from *M. scrofulaceum* results.[16] To verify the correctness of our results, we decided to retrieve one of the two frozen strains and to determine the genetic sequence of the 5′ end of the 16S rDNA. The sequence turned out to be 100% identical to the *M. scrofulaceum* strain sequence that had been deposited in GenBank.

Susceptibility testing was performed on both strains using the macrodilution method in radiometric broth.[17] The two strains appeared to be resistant to amikacin and ciprofloxacin, but were susceptible to rifabutin. Both rifampin and streptomycin appeared to be moderately active. The former strain was susceptible and moderately susceptible to clarithromycin and ethambutol, respectively, while the second strain was moderately susceptible and fully resistant, respectively.

Discussion

Disseminated disease caused by EM reflects impaired host immunity, as illustrated in patients with AIDS.[9] There are also primary immunodeficiencies associated with disseminated EM infection in young children, albeit very rarely;[18] inherited disorders of the IL-12/IFN-γ circuit are more frequent.[19,20] Severe clinical disease caused by EM *M. scrofulaceum* in our patient thus prompted us to search for defects in the IL-12/IFN-γ circuit. As in this case, mycobacterial bone infections in IFN-γR1 deficiency can sometimes mimic the clinical picture of chronic recurrent multifocal osteomyelitis.[21] To our knowledge, this is the first case of disseminated disease caused by *M. scrofulaceum* in a child with a complete IFN-γR1 deficiency.

Complete IFN-γR1 deficiency is autosomal recessive and is associated with complete absence of cell responsiveness to IFN-γ.[19,20] This absence is characteristically associated with severe disseminated mycobacterial infection that may involve the lungs, spleen, liver, lymph nodes, blood, central nervous system, and bones.[22] Onset of acquired mycobacterial infection often occurs before 3 years of age. Infections are typically caused either by EM species acquired from environmental exposure, or by *Mycobacterium bovis* or BCG acquired through vaccination.[22,23] These patients have chronic disease that does not disappear with treatment and that relapses after discontinuing antibiotics. The survival rate is low and 55% of them die before 10 years of age.[22] Bone marrow transplantation should be considered in complete IFN-γR1 deficiency.[24] A recent study showed that the prevalence of mycobacterial disease in patients with IFN-γR1 deficiency was 95% and that the *M. avium* complex was the most commonly isolated mycobacterial pathogen. Overwhelming infection with *Mycobacterium tuberculosis* has also been microbiologically confirmed in a patient already infected with *Mycobacterium fortuitum* and with complete recessive IFN-γR1 deficiency.[22] Disseminated infection with rapidly growing mycobacteria, such as *Mycobacterium smegmatis* and *Mycobacterium peregrinum*, has been reported.[14,25] The present case provides additional evidence that a diagnosis of IFN-γR1 or IFN-γR2 deficiency should be taken into consideration in all children with severe EM disease.

Conflict of interest: No conflict of interest to declare.

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