Paracoccidioides brasiliensis Disseminated Disease in a Patient with Inherited Deficiency in the β1 Subunit of the Interleukin (IL)–12/IL-23 Receptor

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(See the article by Zerbe and Holland on pages e38–41)

Background. Paracoccidioides brasiliensis is a facultative intracellular dimorphic fungus that causes paracoccidioidomycosis (PCM), the most important deep mycosis in Latin America. Only a small percentage of individuals infected by P. brasiliensis develop clinical PCM, possibly in part because of genetically determined interindividual variability of host immunity. However, no primary immunodeficiency has ever been associated with PCM.

Methods. We describe the first patient, to our knowledge, with PCM and a well-defined primary immunodeficiency in the β1 subunit of the interleukin (IL)–12/IL-23 receptor, a disorder previously shown to be specifically associated with impaired interferon (IFN)–γ production, mycobacteriosis, and salmonellosis.

Results. Our patient had a childhood history of bacille Calmette-Guérin disease and nontyphoid salmonellosis and, at the age of 20 years, presented to our clinic with a disseminated (acute) form of PCM. He responded well to antifungal treatment and is now doing well at 24 years of age.

Conclusions. This unique observation supports previous studies of PCM suggesting that IL-12, IL-23, and IFN-γ play an important role in protective immunity to P. brasiliensis. Tuberculosis and PCM are thus not only related clinically and pathologically, but also by their immunological pathogenesis. Our study further expands the spectrum of clinical manifestations of inherited defects of the IL-12/IL-23–IFN-γ axis. Patients with unexplained deep fungal infections, such as PCM, should be tested for defects in the IL-12/IL-23–IFN-γ axis.

During the past 10 years, the molecular basis of the syndrome of Mendelian susceptibility to mycobacterial disease (MIM209950) was determined in a number of patients [1–3]. Mutations of the genes encoding the ligand-binding chain (R1) [4, 5] and associated chains (R2) [6] of the IFN-γ receptor, the β1 subunit of the IL-12 receptor (IL-12Rβ1) [7, 8], the p40 subunit of IL-12 (IL-12p40) [9], and signal transducer and activator of transcription type 1 (STAT-1) [10, 11] have been recognized. The severity of clinical disease was found to correlate with the extent of failure to either produce or respond to IFN-γ. Patients with a complete deficiency of IFN-γR1, IFN-γR2, or STAT-1 lack cellular responses to IFN-γ and have early-onset and life-threatening infections caused by poorly pathogenic mycobacteria and salmonellae. Milder and often curable diseases due to these pathogens are seen in patients with partial IFN-γR1, IFN-γR2, and STAT-1 deficiencies and in patients who lack IL-12p40 (shared by IL-12 and IL-23; hereafter, “IL-12/IL-23p40”) or IL-12Rβ1 (shared by the IL-12 and IL-23 receptors; hereafter, “IL-12/IL-23Rβ1”).

The latter 2 disorders result in normal responses to IFN-γ but abnormal IL-12–dependent and IL-23–dependent production of IFN-γ. Up to 19 patients with IL-12/IL-23p40 deficiency [3, 9, 12, 13] and 54 with IL-12/IL-23Rβ1 deficiency [7, 8, 13–20] have been described. Salmonellosis, another well-known feature of the syndrome of Mendelian susceptibility to mycobacterial disease [21, 22], is particularly common in pa-
Figure 1. Radiograph showing the sequelae of Salmonella enterica serotype Typhimurium osteoarticular infection. The radiograph shows extensive destruction of the head and neck of the femur and also of the acetabulum of the ileum.

tients with IL-12/IL-23p40 and IL-12/IL-23Rβ1 deficiency [3, 23]. Additional unusual infectious diseases have not been reported.

Paracoccidioidomycosis (PCM) is a deep mycosis caused by the dimorphic fungus Paracoccidioides brasiliensis, which is endemic in certain regions of South America [24]. P. brasiliensis naturally undergoes a complex transformation from inhaled environmental conidia into the pathogenic yeast form in the human lungs. According to the current classification, 2 main clinical forms of PCM are distinguished: the acute or juvenile form (AF) and the chronic or adult form (CF) [25]. The CF affects mainly males, who show a high frequency of pulmonary, skin, and mucosal involvement. The lesions affect only few tissues/organs and are associated with tuberculoid granulomas containing a small number of fungi [26]. The AF is characterized by the widespread involvement of the reticuloendothelial system, including lymph nodes, spleen, liver, and bone marrow. The lesions are disseminated and associated with necrotizing host cells and abundant fungal cells.

An intriguing feature of P. brasiliensis infection is that not all infected individuals develop disease. In areas of endemicity in Brazil, P. brasiliensis infects 10%–40% of the population, as detected by serological testing, whereas the incidence of CF and AF PCM is probably less than 1% and 0.1% of infected individuals, respectively. Interestingly, patients with HIV infection are more prone to develop a severe form of PCM, with features of the 2 polar forms of the disease, mainly due to reactivation of latent foci but often resembling the AF of PCM [27]. Nevertheless, despite the increasing number of known primary immunodeficiencies and their improved diagnosis in Brazil, no patient with PCM associated with primary immunodeficiency was reported in the medical literature. This leaves open the question of whether a genetic predisposition may account for PCM clinical disease in the general population. Herein, we describe the first patient with clinical PCM disease and a primary immunodeficiency affecting the IL-12/IL-23–IFN-γ axis.

CASE REPORT

Our patient is a 24-year-old man of Portuguese descent. He is the first son of a nonconsanguineous couple and was born in a small city in the inlands of São Paulo State, Brazil. After bacille Calmette-Guérin (BCG) vaccination as a newborn, he presented to the hospital at 7 months of age with a cervical adenopathy caused by Mycobacterium bovis BCG. The infection resolved after a 6-month course of rifampin, isoniazid, and ethambutol. At 2 years of age, he presented with relapses of lymphadenitis, which responded only partially to multiple antibiotic treatments. At the age of 6 years, disseminated disease caused by Salmonella enterica serotype Typhimurium was diagnosed with multiple lymphadenitis, arthritis of the right hip, and osteomyelitis of the right ilium and femur. This infection...
lasted 7 years and led to osteoarticular sequellae (figure 1). At 20 years of age, after a period of 7 years without symptoms, he developed persistent fever and abdominal pain with disseminated lymphadenopathy and hepatosplenomegaly (figure 2). Biopsy of an abdominal lymph node showed a juvenile (acute) form (AF) of paracoccidioidomycosis, supported by high titers of serum antibodies to *P. brasiliensis* antigens (figure 3). The infection was controlled by trimethoprim-sulfamethoxazole (160 mg trimethoprim and 800 mg sulfamethoxazole twice per day). At the time of writing, the patient is 24 years of age and is healthy after completion of a 5-year course of therapy.

Findings of laboratory analysis conducted during AF PCM showed mild leukopenia (3400 cells/mm³) and moderate lymphopenia (600 cells/mm³); normal serum IgM levels (41 mg/dL), low serum IgA and IgG levels (37 mg/dL and 533 mg/dL, respectively), and elevated IgE levels (383 IU/L); test results that were positive for IgG antibody to cytomegalovirus and negative for IgM antibody to cytomegalovirus, rubella, and *Toxoplasma gondii*; and serological test results that were negative for Epstein-Barr virus and positive for *P. brasiliensis*. Lymphocyte phenotyping showed depletion of CD4+ T cells before and after treatment of PCM (figure 4). Evaluation of the lymphoproliferative capacity of the patient’s T lymphocytes before therapy showed normal stimulation indexes for phytohemagglutinin and pokeweed mitogen and a decreased stimulation index for the anti-CD3 monoclonal antibody (figure 5). In contrast, the antigen-specific T cell proliferation in vitro was depressed for all of the following antigens that were tested: *Candida* metabolic antigen (CMA), tetanus toxoid, *Mycobacterium tuberculosis* purified protein derivative, and the 43-kD glycoprotein from *P. brasiliensis* (gp43). Improvement of the antigen-specific responses was verified after initiation of treatment, revealing a normal stimulation index for CMA. The rate of IL-2 secretion induced by phytohemagglutinin and CMA and gp43 antigens was low, and the rate of IFN-γ secretion induced by CMA and gp43 was high (figure 6).

A mutation in the gene encoding IL-12Rβ1 was suspected by single-strand conformational polymorphism and was identified as a homozygous missense mutation resulting in substitution of leucine for phenylalanine at amino acid 77 [17]. The mutation is recessive and associated with loss of function resulting in complete IL-12/IL-23Rβ1 deficiency, with no detectable surface expression of the receptors. The patient’s parents are heterozygous for this mutation. One of his 2 siblings,

Figure 2. Axial CT of the abdomen during *Paracoccidioides brasiliensis* disseminated infection. It is important to note the extensive intra-abdominal lymphadenomegaly.
Figure 3. Histopathologic characteristics of an affected lymph node biopsy specimen. Top, Hematoxylin-eosin–stained specimen showing granuloma with extensive areas of necrosis (original magnification, ×100). Bottom, Gromott-stained specimen showing multiple fungal structures inside the granuloma, with characteristic budding (arrows; original magnification, ×400).

20-year-old brother, is heterozygous for the gene encoding IL-12Rβ1, and the other, a 14-year-old sister, has 2 wild-type IL12RB1 alleles [17]. Both siblings were vaccinated with BCG without adverse reaction and, at the time of writing, are healthy.

DISCUSSION

We herein describe the first patient with PCM disease and a well-defined primary immunodeficiency—inherited IL-12/IL-23Rβ1 deficiency. This is also the first patient from a PCM-endemic country to be described with a defect of the IL-12/IL-23–IFN-γ axis. This association may be coincidental, because this is the first and only known case of PCM associated with a defect in the IL-12/IL-23–IFN-γ axis. Moreover, although the patient developed an acute (disseminated) form of PCM, there was a prompt and full response to therapy with oral trimethoprim-sulfamethoxazole, which is usually indicated
for milder cases of PCM. On the other hand, the characteristics of *P. brasiliensis* infection suggest that PCM in our patient was not fortuitous but, rather, a consequence of the IL-12/IL-23Rβ1 defect. Indeed, there is a striking clinical and histological resemblance between PCM and mycobacterial diseases, particularly tuberculosis [29]. Although phylogenetically distant, the infectious agents of this 2 diseases invade the host via the respiratory tract, persist within macrophages, cause granuloma formation, and disseminate within the reticuloendothelial system. This study illustrates the importance of the microbial environment in the clinical presentation of primary immunodeficiencies [30].

The studies of IFN-γ knockout mice established the crucial role of IFN-γ in PCM [31]. This research showed that IFN-γ is essential for the resistance and survival of *P. brasiliensis*-infected mice. Furthermore, mice deficient in IFN-γ receptor were also highly susceptible to *P. brasiliensis* intratracheal infection, with increased morbidity and mortality [32]. It is interesting that dissemination of the infection was not observed in association with murine deficiencies in IFN-α or IFN-β receptors [33]. IL-12 knockout mice also demonstrated that IL-12 is of paramount importance in host defense against *P. brasiliensis* [34]. Our present study is thus consistent with the findings in animal models of PCM, which, in turn, suggest that the association of human IL-12Rβ1 deficiency and PCM is not fortuitous.

Patients with PCM often show a suppression of IFN-γ secretion in response to *P. brasiliensis* antigens, contributing to the inability to restrict the dissemination of *P. brasiliensis* [35]. The importance of these immune functions is underscored by the potent secretion of IFN-γ depicted by healthy sensitized subjects who live in areas of endemicity and have positive paracoccidioidin skin test results. As a result, these individuals probably develop an efficient immune response that prevents the onset of the disease. Previous studies showed a preferential secretion of IL-4, IL-5, and IL-10 in patients with AF PCM [36]. These mediators associated with low IFN-γ levels were correlated with a more severe manifestation of the disease. Intermediate immune responses were observed in patients with CF PCM, whose IFN-γ and IL-10 production did not differ from that observed in the group with AF PCM, although IL-4 and IL-5 levels were significantly lower.

Furthermore, in our laboratory, G. Benard and colleagues demonstrated that patients with either AF or CF PCM showed diminished IL-12 secretion in response to gp43, the main *P. brasiliensis* antigenic component [37]. Addition of IL-12 markedly enhanced the mean rate of gp43-elicited IFN-γ secretion by PBMCs. The addition of IL-2 resulted in an additional increase in the IFN-γ production [38], probably owing to the fact that IL-2 is crucial for the persistence of the IL-12Rβ2 subunit after peptide stimulation of T cells through T cell receptor [39]. Indeed, lymphocytes exposed to gp43 obtained from patients with PCM express very low levels of the β2-subunit, compared with cured patients (C. C. Romano and G. Benard, personal communication). Our patient did not secrete high levels of IL-10, showing a selective depression of IL-12 responsiveness without an increase of IL-4 and IL-10. This
finding could be related to a possible minor role played by IL-10, instead of IFN-γ, in the control of PCM.

In conclusion, the present case report emphasizes that the diagnosis of defects of the IL-12–IFN-γ axis should not only be considered for patients with mycobacterial and/or Salmonella infection, but also for patients presenting with PCM or other deep mycoses. This assumption can be emphasized by the fact that an article in this issue describes an autosomal dominant negative IFN-γ receptor in patients with disseminated histoplasmosis [40]. Histoplasma and Paracoccidioides organisms are taxonomically closely related and even belong to the same family—Onygenaceae. Their differences lie in the genus: Ajellomyces (Histoplasma) and Paracoccidioides. Therefore, patients who present with severe or refractory systemic mycoses may have defects in the genes of the Mendelian susceptibility to mycobacterial disease group and should be investigated for inherited disturbances of the IL-12/IL-23–IFN-γ axis.

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References


Figure 6. Production of cytokines (after 6 months of antifungal therapy and clinical remission of the infection) in response to stimulation with phytohemagglutinin for 24 h, Candida metabolic antigen (CMA) for 72 h, and the 43-kD glycoprotein from Paracoccidioides brasiliensis (gp43) for 72 h. PBMCs obtained from 1 patient (black circles) and 10 control subjects (box plots) were stimulated in culture, and supernatants were assessed for IL-2, IFN-γ, IL-4, and IL-10. Boxes, interquartile ranges; horizontal lines within boxes, median values; whiskers, maximum and minimum values.


