Interleukin-12 Receptor β1 Deficiency Predisposing to Disseminated Coccidioidomycosis

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Coccidioidomycosis is caused by the thermally dimorphic molds *Coccidioides immitis* and *Coccidioides posadasii*, fungi endemic to the semi-arid regions of the southwestern United States, Mexico, and parts of South America. Symptomatic disease occurs in only approximately one-third of exposed individuals, manifesting primarily as a pneumonic process [1]. Disseminated disease is estimated to occur in <5% of symptomatic individuals and <1% of all infections, but the reasons are heretofore unknown. Individuals of Filipino, African American, or Hispanic ancestry; women in the third trimester of pregnancy; patients with advanced human immunodeficiency virus (HIV) infection; and transplant recipients are at increased risk of disseminated disease [2]. The latter 3 risk groups implicate cell-mediated immunity in the control of *Coccidioides* species, and the ethnic associations suggest significant genetic contributions to coccidioidomycosis susceptibility. We recently described a young man with chronic progressive coccidioidiomycosis and disseminated *Mycobacterium kansasii* infection who had an 818del4 mutation in *IFNGR1*, resulting in dominant partial deficiency of the interferon (IFN)–γ receptor [3]. We report 2 related patients with disseminated coccidioidomycosis with a novel missense mutation in the β1 subunit of the interleukin (IL)–12 receptor (*IL12RB1*) C186Y. This confirms the importance of the IL-12/IL-23/IFN-γ pathway in the control of this fungal infection.

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PATIENT 1

This 22-year-old woman presented in February 2008 with a 4-day history of retrosternal discomfort, dysphagia, and odynophagia, in addition to a 2-day history of subjective fevers and chills. CT revealed mediastinal and right supraclavicular lymphadenopathy. *Coccidioides* IgM and IgG serologic findings were positive. She was treated with fluconazole for 3 months and then was lost to follow-up. She presented again in October 2008 with a 1-month history of fever, chills, non-productive cough, and diffuse body aches. CT demonstrated lymphadenopathy in the cervical, supraclavicular, hilar, mediastinal, retroperitoneal, and upper abdominal areas. Cervical lymph node biopsy showed non-necrotizing granulomata with abundant coccidioidal spherules (Figure 1A). She was treated with fluconazole for ~1.5 years, with resolution and without recurrence.

The family originates from Palestine, and her parents are first cousins. Her medical history is significant for a febrile illness at age 11 years that developed while traveling in the Middle East and was associated with cervical lymphadenopathy that progressively enlarged over a 1-month period. Lymph node biopsy and repeated blood cultures yielded *Salmonella* serogroup D. There was no other focus of infection (eg, osteomyelitis) by imaging. Cure was eventually achieved after 6 weeks of intravenous antibiotic therapy.

PATIENT 2

The 18-year-old younger brother of patient 1 was born in Phoenix, Arizona. At age 6 years, he was hospitalized with fever and cough and received a diagnosis of "valley fever" (acute coccidioidal pneumonia). He improved with fluconazole therapy but was lost to follow-up. At 14 years of age and no longer receiving fluconazole, he developed right supraclavicular lymphadenopathy and a nasal lesion; spherules were seen on biopsy. Fluconazole therapy was given until the age of 16 years. Two months after discontinuation, he developed right knee pain and weight loss without fever or night sweats. A lesion in the right proximal tibia showed granulomatous osteomyelitis with spherules; cultures grew *Coccidioides* species. Itraconazole led to improvement. He has no other history of infection.

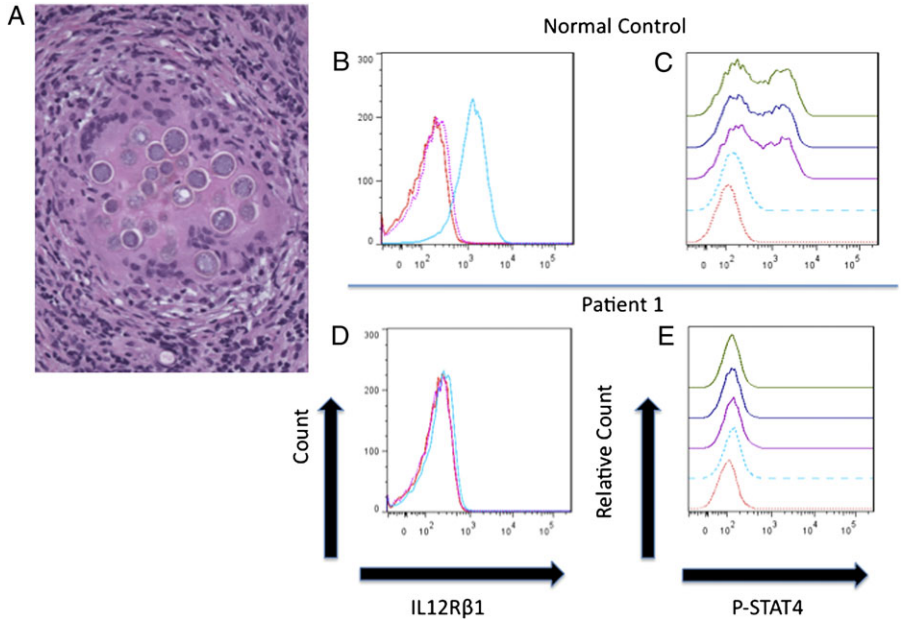

Figure 1. *A*, Abundant *Coccidioides* species spherules in lymph node. *B*, Normal activated T cells showing robust cell surface display of IL12Rβ1. *C*, Offset histograms showing progressive STAT4 phosphorylation in response to added IL-12 (isotype control [bottom], 0, 2.5, 5, and 10 [top] ng/mL, respectively). *D*, Activated T lymphocytes from patient 1 failed to express IL-12Rβ1 on the cell surface. *E*, Phosphorylation of STAT4 is absent in lymphocytes from patient 1 at the same IL-12 doses used in *C*.

Both patients tested negative for HIV on multiple occasions; demonstrated no quantitative abnormality in peripheral blood $CD14^+$ monocytes, $CD3^+$ T cells and subsets, $CD20^+$ B cells, and $CD16^+$ natural killer (NK) cells; and had no evidence of elevated serum IgM level or hypogammaglobulinemia.

Because of extra-intestinal salmonellosis and subsequent extrathoracic coccidioidomycosis in patient 1, an immunological deficiency involving IL-12 was pursued. Peripheral blood mononuclear cells (PBMCs) demonstrated significantly reduced production of IFN-γ after stimulation with phytohemagglutinin (PHA) and failure to augment production in response to PHA with IL-12, suggesting a defect involving the IL-12 receptor. Sequencing of *IL12RB1* confirmed homozygous 557G > A transition, resulting in a cysteine to tyrosine substitution at amino acid position 186 (C186Y). A distinct pathologic mutation at this same amino acid position (C186S) resulting in salmonellosis but variable susceptibility to *Mycobacterium bovis*–bacilli Calmette Guérin has been previously reported [4].

Epstein Barr virus–transformed B cells and T lymphocytes activated with PHA and IL-2 from patient 1 lacked IL12Rβ1 on the cell surface with use of commercially available antibodies (BD Pharmingen, clone 2.4E6; R&D, clone 69310) (Figure 1B and D), although protein was detectable on intracellular staining with use of 2.4E6 (data not shown). Despite the presence of intracellular IL12Rβ1, the patient's cells failed to respond to escalating doses of IL-12, as demonstrated by the absence of phosphorylation of signal transducer and activator of transcription–4, confirming a complete functional deficiency of the IL-12 receptor (Figure 1C, and E).

Buccal cells from patient 2 demonstrated the same homozygous mutation as that in patient 1, and both unaffected parents were heterozygous. The unaffected sibling was not tested.

DISCUSSION

It has long been appreciated that disseminated coccidioidomycosis occurs in only a small minority of those at risk. These 2 cases are typical of disseminated disease. In conjunction with our previous report of disseminated coccidioidomycosis in a patient with a dominant IFN-γ receptor mutation, this shows the centrality of the IL-12/IL-23/IFN-γ axis to human control of *Coccidioides* species.

Early work established that IFN-γ was required for macrophage killing of phagocytosed *Coccidioides* species and that this process was potentiated by IL-12 [5, 6]. Subsequently, IL-12 and IFN-γ responses of human PBMCs stimulated in vitro with coccidioidal antigens were found to distinguish immune (delayed type hypersensitivity–positive) from nonimmune (delayed type hypersensitivity –negative) donors [7]. Specifically, PBMCs from nonimmune donors with disseminated coccidioidomycosis produced significantly less IFN-γ than did those from healthy immune donors. Furthermore, although IFN-γ production by PBMCs from immune donors could be dramatically increased by IL-12, no increase was seen

in patients with disseminated coccidioidomycosis, and this hypo-responsiveness to exogenous IL-12 in nonimmune patients was associated with impaired activation of STAT4 [7]. These studies unequivocally established IL-12 and IFN-γ as key mediators of cellular immunity to *Coccidioides* species, and the cases that we report prove that mutations in the IL-12/IL-23/IFN-γ axis can confer susceptibility to disseminated coccidioidomycosis. It will be interesting to determine whether functional polymorphisms in genes of this axis may account for the ethnocentric vulnerability described in epidemiological studies.

Coccidioides species share the essential property of thermal dimorphism with only a select few other human pathogenic fungi, namely, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Blastomyces dermatitidis*, *Penicillium marneffei*, and *Sporothrix schenkii*. Despite differences in phylogeny, biology, and almost certainly in pathogen-associated molecular patterns, human immunological responses converging on the IL-12/IL-23/IFN-γ axis appear to be fundamentally necessary for protection from some of these dimorphic moulds, because mutations in this pathway have also been identified in cases of disseminated histoplasmosis and paracoccidioidomycosis [8, 9]. Of interest, disseminated coccidioidomycosis and histoplasmosis have been described in patients with autosomal dominant hyper-IgE (Job) syndrome due to mutations in STAT3. Although the mechanism conferring susceptibility remains undefined, STAT3 is critical for IL-23 signaling, and IL-23 may be relevant for synergistic induction of IFN-γ and IL-12 production [10]. Collectively, these genetic immunodeficiencies illustrate the importance of this axis to control these dimorphic fungi. As a corollary, patients with refractory or disseminated disease with these dimorphic fungi should be evaluated for functional defects in these pathways.

The cases described here are distinct because of their predominant presentations with deep mycoses. Defects in the IL-12/IL-23/IFN-γ axis have been collectively referred to as Mendelian susceptibility to mycobacterial disease (MSMD), based on the seminal discoveries that individuals harboring such mutations were susceptible to these pathogens [11]. However, the susceptibility phenotype of these genetic disorders continues to expand and now includes thermally dimorphic fungi. In contrast to previous MSMD reports, neither of the patients here has developed mycobacterial infection. Because nontuberculous mycobacteria are thought to be ubiquitous, it is unclear whether this absence represents a regional variation or an effect of this particular mutation predisposing selectively to moulds. The variability in susceptibility to mycobacteria associated with mutations in IL12Rβ1 has been well described [4], presumably because IL12 is somewhat redundant in protective immunity against mycobacteria. However, the episode of salmonellosis in patient 1 is characteristic of her IL12Rβ1 defect.

Studies of primary immunodeficiencies have defined critical components of natural human immunity to pathogens. Recently, the features of human immunity to fungi have been increasingly characterized. Defects in the phagocyte nicotinamide adenine dinucleotide phosphate oxidase system, which cause chronic granulomatous disease, predispose to infection with *Aspergillus* species and other nondimorphic hyalohyphomycetes (eg, *Fusarium* species, *Paecilomyces* species, and *Penicillium* species). Impairment of the IL-17/IL-22 axis, seen in STAT3 deficiency and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, manifests with mucocutaneous candidiasis [12–14]. It is clear from this and previous reports that the IL-12/IL-23/IFN-γ axis is critical to the control of thermally dimorphic fungi [3, 8, 9]. These primary immunodeficiencies demonstrate that the occurrence of severe fungal disease is not random but can reflect a discrete immune defect. These disorders suggest the immunologic pathways that may be useful to understand susceptibility to invasive fungal disease in at-risk populations.

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