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Inborn errors of immunity underlying fungal diseases in otherwise healthy individuals

Juan Li^{1,*}, Donald C. Vinh^{2,*}, Jean-Laurent Casanova^{1,3,4,5,6}, and Anne Puel^{1,3,4,@}

¹St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, NY, USA

²Infectious Disease Susceptibility Program, McGill University Health Centre (MUHC) and Research Institute-MUHC (RI-MUHC), Montreal, Quebec, Canada

³Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM U1163, Necker Hospital for Sick Children, 75015 Paris, France, EU

⁴Paris Descartes University, Imagine Institute, 75015 Paris, France, EU

⁵Pediatric Hematology-Immunology Unit, Necker Hospital for Sick Children, 75015 Paris, France, EU

⁶Howard Hughes Medical Institute, New York, NY, USA

Abstract

It has been estimated that there are at least 1.5 million fungal species, mostly present in the environment, but only a few of these fungi cause human disease. Most fungal diseases are self-healing and benign, but some are chronic or life-threatening. Acquired and inherited defects of immunity, including breaches of mucocutaneous barriers and circulating leukocyte deficiencies, account for most severe modern-day mycoses. Other types of infection typically accompany these fungal infections. More rarely, severe fungal diseases can strike otherwise healthy individuals. Historical reports of fungi causing chronic peripheral (e.g. affecting the nails, skin, hair) infections, and invasive disease (e.g. brain, lungs, liver), in otherwise healthy patients, can be traced back to the mid-20th century. These fungi typically cause endemic, but not epidemic diseases, are more likely to underlie sporadic than familial cases, and only threaten a small proportion of infected individuals. The basis of this “idiosyncratic” susceptibility has long remained unexplained, but it has recently become apparent that “idiopathic” fungal diseases, in children, teenagers, and even adults, may be caused by single-gene inborn errors of immunity. The study of these unusual primary immunodeficiencies (PIDs) has led to the identification of molecules and cells playing a crucial role in human host defenses against certain fungi at particular anatomic sites. A picture is emerging of inborn errors of IL-17 immunity selectively underlying chronic mucocutaneous candidiasis, with little inter-individual variability, and of

@corresponding author: anne.puel@inserm.fr.

*equal contributions

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inborn errors of CARD9 immunity underlying various life-threatening invasive fungal diseases, differing between patients.

Introduction

Chronic or life-threatening fungal diseases typically occur in patients with overt immunodeficiencies (IDs), whether inherited or acquired [1,2]. They represent a major public health problem, with a growing population of patients at risk, due to increases in the frequency of invasive procedures, cancers, chemotherapy, and immunosuppressive treatments, and the aging of the population, together with the emergence of antifungal drug-resistant strains. These factors result in various types of breaches of the mucocutaneous barrier and deficiencies of circulating leukocytes. Fungal diseases are responsible for considerable morbidity and mortality worldwide [3,4]. Their pathogenesis remains poorly understood, because they frequently develop in patients with multiple morbidities, and, therefore, with several risk factors. For the same reason, these patients typically suffer from a number of other infections. However, in rare cases, severe fungal diseases can strike otherwise healthy patients with no known underlying risk factors [5]. These “idiopathic” fungal diseases paradoxically provide us with a unique opportunity to gain insight into the general mechanisms of their pathogenesis.

It has recently become apparent that “idiopathic” fungal diseases may be caused by single-gene inborn errors of immunity [6,7]. A first hint that this might be the case was provided by the observation that such diseases affect a very small minority of infected individuals, whereas the diseasing-causing fungi are ubiquitous in the environment [2,8]. Some well-characterized primary immunodeficiencies (PIDs), such as congenital neutropenia and chronic granulomatous diseases, underlie various infections, including relatively specific sets of fungal diseases [9]. In addition, it is becoming increasingly clear that a number of viral and bacterial diseases striking otherwise healthy individuals result from single-gene inborn errors of immunity [10–13]. In this context, several fungal diseases, including chronic mucocutaneous candidiasis, *Candida* meningoencephalitis, deep dermatophytosis, and subcutaneous or invasive phaeohyphomycoses, have recently been shown to be caused by inborn errors of immunity [7,14,15].

The identification and characterization of genetic and immunological defects underlying fungal diseases can shed new light on the molecular and cellular mechanisms conferring protective immunity to specific fungi, by clarifying the pathogenesis of the corresponding fungal diseases in patients [16]. This work has important clinical implications, as it makes it possible to provide patients and their families with molecular genetic diagnoses and genetic counseling. Moreover, these advances pave the way for novel prophylactic or curative therapeutic interventions, both in the setting of these inherited IDs and in patients with these fungal diseases in the context of other, acquired IDs, based on a rational understanding of pathogenesis. Research into the human genetic basis of fungal diseases is important and timely, given the rapid emergence of antifungal drug-resistant strains and the high mortality rate associated with fungal diseases, despite appropriate antifungal treatment [2].

Chronic mucocutaneous candidiasis: inborn errors of IL-17 immunity

Chronic mucocutaneous candidiasis (CMC) is characterized by severe, persistent or recurrent infections of the mucosa, skin, and/or nails with *Candida* spp. CMC is common in patients with acquired or inherited profound T-cell immunodeficiency, who usually display multiple other, potentially more serious, infectious and/or autoimmune diseases [14]. CMC can also be syndromic in some PIDs without overt global T-cell deficiencies. These PIDs include autosomal dominant (AD) hyper-IgE syndrome (HIES) caused by dominant negative *STAT3* loss-of-function (LOF) mutations, AD *STAT1* gain-of-function (GOF), autosomal recessive (AR) autoimmune polyendocrinopathy syndrome type 1 (APS-1) caused by biallelic mutations of *AIRE*, and AR caspase recruitment domain-containing protein 9 (CARD9), IL-12 receptor $\beta 1$ (IL-12R $\beta 1$), IL-12p40 or ROR γ / γ T deficiencies [17]. These patients generally display fewer other infectious and/or autoimmune manifestations than patients with severe combined immunodeficiencies (SCID) or combined immunodeficiencies affecting T-cell numbers or functions, and CMC is one of the main clinical presentations. An analysis of the molecular and cellular basis of CMC in these PIDs has suggested a possible role of IL-17-mediated immunity in protection against mucocutaneous candidiasis [18]. Indeed, all these PIDs have CMC as a common clinical phenotype, and all are characterized by abnormally low proportions of circulating IL-17⁺ T cells, or high serum levels of neutralizing autoantibodies against IL-17 cytokines (in the case of patients with AR APS1). The low proportions of circulating IL-17⁺ T cells result from impaired pro-Th17 cytokine signaling or production (e.g. AR IL-12p40 and IL-12R $\beta 1$ deficiencies, impairing IL-23 production and response, respectively; AD *STAT3* deficiency, impairing signaling downstream from IL-6, IL-23, and IL-21, in particular) [19]; the lack of a master transcription factor for IL-17⁺ T cells and type 3 innate lymphoid cells (AR ROR γ T deficiency) [20]; an increase in signaling downstream from cytokines inhibiting IL-17⁺ T-cell differentiation (AD *STAT1* GOF) [21]; and the impaired production of pro-Th17 cytokines by phagocytes upon fungal recognition (AR CARD9 deficiency) [22]. These studies have paved the way for the identification of inborn errors of immunity conferring CMC in otherwise healthy individuals [18].

Autosomal dominant IL-17F deficiency

AR IL-17RA deficiency, and AD IL-17F deficiency were identified (after the Sanger sequencing of candidate genes) in 2011, as the first genetic causes of isolated inherited CMC [23]. The heterozygous missense mutation (S65L) of the *IL17F* gene was identified in five patients from an Argentinian multiplex family with early-onset CMC (within the first year of life), and two asymptomatic family members (indicating incomplete clinical penetrance) [23]. The index patient was also reported to have had recurrent upper respiratory tract infections, asthma, and recurrent episodes of furunculosis since infancy. These patients had normal proportions of IL-17A- and IL-22-producing T cells, but it was not possible to evaluate the proportion of IL-17F-expressing T cells by flow cytometry, even for control cells [23]. The S65L mutation did not affect the production of wild-type or mutant IL-17F monomers or homodimers or of IL-17A/IL-17F heterodimers. Instead, it affected the binding of the mutant S65L IL-17F protein to the IL-17RA/IL-17RC receptors expressed on the surface of control fibroblasts [23]. Accordingly, control fibroblasts and keratinocytes

displayed impaired responses to mutant IL-17F homodimers, and to wild-type IL-17A/mutant IL-17F and wild-type IL-17F/mutant IL-17F heterodimers [23]. Heterodimers of the mutant IL-17F protein with either wild-type IL-17F or IL-17A exerted a dominant-negative effect on IL-17A- or wild-type IL-17F-mediated responses. The S65L IL-17F mutation is, therefore, a hypomorphic dominant-negative mutation [23]. A heterozygous *IL17F* mutation was subsequently reported in a woman and her son of Tunisian-German origin with early childhood onset of AD CMC, in the absence of any other infectious phenotype, including staphylococcal disease in particular [24]. This mutation has not yet been characterized, but correspond to the second case of AD IL-17F deficiency. These findings strongly suggest that AD IL-17F deficiency underlies isolated CMC in humans.

Autosomal recessive IL-17RA deficiency

The discovery of AR IL-17RA deficiency, described in the same report in 2011, provided the most direct evidence to date for an essential role of IL-17 immunity in protection against mucocutaneous *Candida* infections [23]. The patient, born to consanguineous Moroccan parents, presented early-onset (at one month) CMC and cutaneous *Staphylococcus aureus* infection (at five months), and was homozygous for a nonsense (Q284*) mutation of *IL17RA*. A large homozygous deletion encompassing *IL17RA* and *ADA2*, encoding adenosine deaminase 2, was subsequently identified in two siblings from Sri Lanka with CMC, *S. aureus* skin infection, and chronic inflammation [25]. Since 2011, AR IL-17RA deficiency, resulting from diverse homozygous nonsense (R66*, Q86*, Q284*, and Y384*), missense (D387N), frameshift indel (H38Afs*15, C57Yfs*5, L90Cfs*30, P257Rfs*16, N440Rfs*50, and Y591Sfs*29), splice site (c.163+1G>A) mutations, and large deletions (770 kb at *22q11.1*), has been reported in 23 patients from 13 unrelated kindreds originating from Morocco, Turkey, Japan, Saudi Arabia, Algeria, Argentina, and Sri Lanka [25,26]. All patients displayed early-onset (before 6 months of age) CMC affecting mucosal sites (oral thrush and anogenital candidiasis), the skin, scalp, and nails. In addition, 16 of these patients presented staphylococcal skin diseases (abscesses, folliculitis, furunculosis, and crusted pustules on the face and scalp, sometimes spreading to the shoulders and arms), and 10 patients were prone to recurrent infections of the respiratory tract, including otitis, sinusitis, bronchitis, and lobar pneumonia [25,26]. Consistent with the first report, fibroblasts derived from the skin of the patients did not respond to IL-17A and IL-17F homodimers and heterodimers, whereas no response to IL-25 was detected in their leukocytes, including cells from the patient carrying the only missense D387N mutation with a receptor detectable by flow cytometry at the surface of fibroblasts and monocytes [23,26,27]. We can conclude that IL-17RA plays an essential role in mucocutaneous immunity to *C. albicans* and, to a lesser extent, to *S. aureus*, but that it is probably otherwise redundant in host defense (Figure 1, Table 1).

Autosomal recessive IL-17RC deficiency

In 2015, AR IL-17RC deficiency was discovered, by whole-exome sequencing, in three unrelated patients with isolated early-onset (2 months to 4 years) CMC, two of whom were born to consanguineous parents from Turkey and Argentina [28]. Unlike patients with AR IL-17RA deficiency, these patients did not seem to suffer from *S. aureus* skin infections. Surface IL-17RA expression was normal in the patients' fibroblasts, but all the homozygous

nonsense (Q138*, R376*, and R378*) *IL17RC* mutations identified were shown, by overexpression, to be loss-of-expression, leading to the absence of IL-17RC at the cell surface [28]. In both mice and humans, IL-17RA and IL-17RC are required for IL-17A and IL-17F signaling [29]. As expected, like the fibroblasts of patients with AR IL-17RA deficiency, fibroblasts derived from patients with AR IL-17RC deficiency did not respond to IL-17A and IL-17F homodimers and heterodimers [28]. However, unlike PBMCs from patients with AR IL-17RA deficiency, IL-17RC-deficient PBMCs responded normally to IL-25 [28]. Impaired responses to IL-25 or, perhaps, to other IL-17RA-dependent cytokines may account for the staphylococcal diseases frequently seen in patients with AR IL-17RA deficiency, but not in patients with AD IL-17F or AR IL-17RC deficiencies. Finally, whole blood from patients with AR IL-17RA or IL-17RC deficiency displays normal IL-6, IFN- γ , and IL-17A production, and normal TNF- α monocyte-derived DC (MDDC) production in response to fungal compounds [26,28]. These data strongly suggest that defective IL-17RA- and IL-17RC-dependent signaling is primarily responsible for CMC, whereas the production of IL-17A is normal in response to stimulation with *C. albicans*. In summary, human IL-17RC deficiency provides further evidence for the requirement of IL-17 immunity for host defense against *C. albicans* mucocutaneous infection, and its redundancy against most other common microbes (Figure 1, Table 1).

Autosomal recessive ACT1 deficiency

AR ACT1 deficiency, resulting from a homozygous missense mutation (T536I) of the *TRAF3IP2* gene, was reported in 2013, in two siblings born to consanguineous Algerian parents presenting with CMC and recurrent *S. aureus* disease of the skin and scalp since early childhood [27]. ACT1 is a key adapter molecule in the IL-17 response pathway. It is recruited to IL-17RA/IL-17RC and IL-17RA/IL-17RB through its SEFIR domain, in response to IL-17A, IL-17F, and IL-25 [29]. ACT1 is also recruited to CD40 and B cell-activating factor of the tumor necrosis factor (TNF) family receptor (BAFF-R) upon CD40 ligand (CD40L) and BAFF stimulation, respectively, possibly as a negative regulator [30]. In fibroblasts derived from patients homozygous for the T536I ACT1 mutation, ACT1 protein levels and subcellular distribution are normal [27]. However, the T536I mutation, located in the SEFIR domain of ACT1, selectively impairs the homotypic interaction of ACT1 with IL-17RA, IL-17RB, and IL-17RC, but not the SEFIR-independent interactions of ACT1 with other proteins, such as CD40, heat shock protein 70 (HSP70), and HSP90 [27]. The patients' fibroblasts and leukocytes thus fail to respond to IL-17A and IL-17F and to IL-25, whereas their B cells respond normally to CD40L [27]. ACT1-deficient mice are prone to oropharyngeal candidiasis [31] and have enhanced B-cell responses to CD40L and BAFF, leading to hypergammaglobulinemia [30]. The selective defect of IL-17 signaling in fibroblasts and leukocytes carrying the ACT1 T536I mutation probably explains the phenotypic discrepancy between patients with *ACT1* mutations and ACT1-deficient mice. Thus, AR ACT1 "deficiency" provides additional evidence for the requirement of IL-17A- and IL-17F-mediated immunity for protection against *C. albicans*. Furthermore, in these two patients with *S. aureus* skin disease, it confirms the role of IL-17RA/ACT1 in protection against cutaneous bacterial infections. The identified genetic etiologies of CMC reveal that IL-17F cytokines and IL-17RA/IL-17RC receptors are essential components of human mucocutaneous immunity to *C. albicans*, but probably otherwise largely redundant in

protection against viruses, bacteria other than *S. aureus*, and fungi other than *C. albicans* (Figure 1, Table 1).

Implications for IL-17 immunity

IL-17A and IL-17F belong to the IL-17 cytokine family, which has four other members: IL-17B, IL-17C, IL-17D, and IL-17E (or IL-25). IL-17A and IL-17F (the IL-17 family member most closely related to IL-17A) are the best characterized to date. In 2005, mouse studies showed that IL-17A and IL-17F, together with other cytokines, such as IL-22, were produced principally by a subset of CD4⁺ T helper cells, the Th17 cells (reviewed in [32,33]). Additional cell types, including $\gamma\delta$ T cells and type 3 innate lymphoid cells (ILC3), have since been identified as sources of IL-17A and IL-17F [32]. IL-17A and IL-17F bind as homodimers or heterodimers to IL-17RA/IL-17RC heterodimeric receptors, which themselves belong to the IL-17R family, which also includes IL-17RB, IL-17RD and IL-17RE (reviewed in [29]). IL-17RA is broadly expressed on cells from both the hematopoietic and non-hematopoietic compartments, whereas IL-17RC expression is mostly restricted to the non-hematopoietic compartment (e.g. epithelial cells) [29]. Furthermore, human IL-17RA binds IL-17A with a much higher affinity than IL-17F, whereas IL-17RC and IL-17RA/IL-17RC have similar affinities for IL-17A and IL-17F [34]. Upon ligand binding, IL-17RA/IL-17RC binds the intracellular adaptor ACT1 to activate the downstream NF- κ B, mitogen-activated protein kinase (MAPK), and CCAAT/enhancer-binding protein (C/EBP) signaling pathways, thereby driving the transcription of target genes, such as those encoding matrix metalloproteinases (e.g. MMP1, MMP3), antimicrobial peptides (e.g. β -defensins, S100 proteins), and pro-inflammatory cytokines and chemokines (e.g. IL-1, IL-6, G-CSF, GM-CSF, CXCL1, CXCL2, CXCL9, CCL20) involved in the recruitment and activation of leukocytes (e.g. neutrophils, Th17 or ILC3) to the site of infection [29,32] (Figure 1). IL-17A is also involved in the stabilization of various mRNAs, including those encoding cytokines and chemokines, through TRAF2 and TRAF5 [29]. IL-17A and IL-17F (in synergy with TNF- α) thus function to protect humans against fungal (*C. albicans*) and bacterial (*S. aureus*) infections of the mucosal and epithelial surfaces (e.g. skin, oral cavity, esophagus or lungs) ([26], reviewed in [14,35]). In this context, almost all patients with inborn errors of IL-17-mediated immunity reported to date, including those with strongly impaired IL-17 production (e.g. heterozygous *STAT3* LOF or *STAT1* GOF mutations, or homozygous *RORC* LOF mutations) display CMC, whereas none of these patients display any other superficial or invasive fungal disease with such high clinical penetrance (Table 1).

Invasive fungal diseases: inborn errors of CARD9 immunity

The discovery of PIDs underlying invasive fungal diseases (IFDs), in patients with or without other infectious diseases, has helped to identify critical pathways in immunity to some IFDs [36]. For example, chronic granulomatous disease (CGD), caused by inherited defects affecting the function of the phagocyte NADPH oxidase complex, is characterized by recurrent life-threatening bacterial and fungal diseases, mostly caused by *Aspergillus* spp. and other hyaline filamentous fungi [37–41]. The phagocyte oxidative burst is, thus, essential for protective immunity to *Aspergillus* spp., particularly in the lungs, but redundant

against other fungi (e.g. *Cryptococcus neoformans*) [38]. However, about two-thirds of CGD patients do not develop diseases caused by *Aspergillus* spp., so other pathways may be sufficient to control these fungi [42,43]. Likewise, Mendelian susceptibility to mycobacterial disease (MSMD), due to mutations affecting the IL-12/IL-23/IFN- γ axis, predisposes individuals not only to infections with *Mycobacteria* spp. and *Salmonella* spp. [44], but also to CMC, and disseminated disease due to thermally dimorphic endemic fungi (e.g. *Coccidioides* spp.; *Paracoccidioides* spp.; *Histoplasma* spp.) [45–50]. Additional examples include *Pneumocystis jirovecii* pneumonia, which is frequent in patients with acquired (HIV) [51] or inherited severe T-cell immunodeficiencies, or patients with severe congenital neutropenia (SCN), who are susceptible to peripheral and invasive infections caused by bacteria and fungi, including *Aspergillus* and *Candida* spp. in particular (reviewed in [7]). In patients with these conditions, isolated fungal infections are rare, the vast majority of fungal infections instead being associated with other microbial infections. By contrast, CARD9 deficiency is a recently described PID involving susceptibility that appears to be restricted to specific fungi (Figure 2) in otherwise healthy individuals, including children, adolescents and, more surprisingly, adults up to the age of about 50 [15].

Autosomal recessive CARD9 deficiency and invasive candidiasis

The seminal discovery of AR CARD9 deficiency was made in a large multiplex consanguineous family from Iran, in which six of the seven patients described suffered from CMC. Four of these patients were genotyped and found to be homozygous for a nonsense (Q295*) *CARD9* mutation [22]. In addition, three relatives died in adolescence from possible *Candida* meningitis/meningoencephalitis [22]. This report identified AR CARD9 deficiency as a possible genetic etiology of CMC. However, CMC has since been described in only 20 of the 53 (37.7%) reported patients with CARD9 deficiency [22,52–58]. It therefore seems that a loss of CARD9 function confers a predisposition to CMC, but with incomplete penetrance. Conversely, AR CARD9 deficiency now appears to be a genetic cause for invasive candidiasis [15]. Since the initial report, the most striking and consistent disease attributed to AR CARD9 deficiency is *Candida* disease of the central nervous system (CNS), affecting the brain parenchyma (abscess) and/or the meninges (meningitis). In the 16 cases of reported with probable or proven *Candida* CNS disease due to CARD9 deficiency [22,52,55,56,58–62], *C. albicans* was the primary agent (one case was due to the phylogenetically related *C. dubliniensis* [59]; another possibly due to *C. glabrata* [52]). The disease presented during childhood or adulthood (mean: 20.0 years; median 17.0 years; range [3.5 to 39 years]). This is intriguing, as colonization with *C. albicans* begins very early in life (e.g. before the age of one year in ~50% of cases) [63]. The presenting symptoms and brain imaging findings are variable, with lesions ranging from abscesses, or solitary masses, to multiple nodules, which may be cystic and even misinterpreted as cancers. Cerebrospinal fluid (CSF) analysis typically reveals a mononuclear cell (lymphocytes and/or mononuclear cells) predominance; eosinophil pleocytosis but no neutrophil pleocytosis in most cases; high protein concentration; and hypoglycorrhachia. Three cases of *C. albicans* osteomyelitis [56,61,64], two of which were associated with endophthalmitis, one case of intra-abdominal (liver and mesenteric lymph nodes) candidiasis [57], and two cases of severe *Candida* colitis [52] have also been reported (Table 2). Three of the 16 patients died [22]. Relapses may occur despite appropriate antifungal therapy (based on *in vitro* antimicrobial susceptibility

testing), even during suppressive (maintenance) treatment, in the absence of antifungal drug resistance [52,58,60,62]. Although *C. albicans* is not known to be neurotropic, invasive candidiasis in CARD9-deficient patients seems primarily to affect the CNS. To date, about 50 cases of 'idiopathic' *Candida* CNS disease have been reported, some of which may be due to CARD9 deficiency [52].

CARD9 deficiency and deep dermatophytic disease

Ubiquitous filamentous dermatophytes typically cause benign superficial disease (e.g. tinea, onychomycosis), but they may also cause deep dermatophytosis, a rare but severe, sometimes life-threatening manifestation, characterized by dermal and subcutaneous tissue invasion and destruction, often with dissemination to lymph nodes (LN), and occasionally, to viscera, bones, and the CNS [65,66]. Deep dermatophytosis has been clinically recognized since at least the 1950s, with investigations suggesting an autosomal recessive pattern of transmission, but the basis of susceptibility to deep dermatophytosis remained unclear until it was discovered that this disease was due to AR CARD9 deficiency in at least some affected individuals, principally from North Africa [53]. To date, 21 patients with AR CARD9 deficiency and extensive or deep dermatophytosis have been reported, mostly originating from North Africa: Algeria ($n=12$), Morocco ($n=2$), Tunisia ($n=4$), Egypt ($n=1$), but also from Turkey ($n=1$) and one patient was a Brazilian born to Italian parents. The male:female ratio for this disease is 15:6 [53,54,58,67,68]. Based on published microbiological data, deep dermatophytosis in these patients was caused principally by *Trichophyton* spp. (*T. violaceum* and *T. rubrum*, $n=14$). Disease onset was typically during childhood (mean age 9 years, median age 8 years, range 2–21 years), with presentation as a superficial disease. However, the dermatophytosis indolently progressed in a destructive manner, over years or decades. Nodular, tumoral, and/or ulcerative lesions developed in adulthood, accompanied by lymphadenopathy and deep organ involvement (e.g. bones, viscera, CNS) (Table 2). Four of the 21 patients died with clinically active deep dermatophytosis, despite antifungal treatment in three of these cases. Treatment is difficult, with recalcitrance to antifungal induction therapy and/or relapse following the cessation of antifungal treatment. All cases of deep dermatophytosis reported to date were found to be caused by AR CARD9 deficiency, suggesting a rare example of genetic homogeneity in an infectious disease.

CARD9 deficiency and phaeohyphomycoses

Phaeohyphomycetes are dematiaceous (melanin-pigmented) fungi responsible for superficial (i.e. chronic cutaneous/subcutaneous, corneal) or systemic diseases. On the basis of histological findings, they can be differentiated into eumycetoma, chromoblastomycosis, and phaeohyphomycosis [69]. These fungi have a worldwide distribution, and are found in soil, wood, and decomposing plant debris. Most reported cases of human disease occur in tropical and subtropical areas, and are due to *Exophiala* spp., *Phialophora* spp., and *Alternaria* spp. [69]. The first indication of a critical role for CARD9 in immunity to phaeohyphomycetes was provided by the identification of biallelic *CARD9* mutations in four Chinese patients (mean age: 21.8 years; median age: 16.5 years, range: 6–48 years) with disfiguring subcutaneous disease due to *Phialophora verrucosa*, a weakly pathogenic fungus, including one patient with a contiguous extension to the eye [70] (Table 2). Infection with *P. verrucosa*

usually follows the contamination of traumatic skin lesion with soil debris, typically on exposed body parts (e.g. legs, hands, arms, face, neck, and back). However, progressive destructive disease due to *P. verrucosa* in patients with no known underlying IDs remains extremely rare (28 cases from Japan, China, India, the Philippines, Korea, Libya, Morocco, Mexico, Brazil, with a mean age at onset of 40 years [6–82 years], have been reported). *CARD9* deficiency was also identified in two patients with systemic phaeohyphomycosis due to *Exophiala* spp. [71]. The first patient, born to Angolan parents in France, suffered from liver and brain disease due to *E. dermatitidis* at the age of five years. The other patient, a woman born to consanguineous Iranian parents, suffered from *E. spinifera* infection with peri-orbital, musculoskeletal, lung, and LN involvement, from the age of 18 years (Table 2). Invasive infections caused by *E. (Wangiella) dermatitidis* or *E. spinifera* are rare (fewer than 80 cases reported worldwide), and about 35 cases (mostly caused by *E. dermatitidis*) with no known underlying risk factors have been reported [72]. Historical reports of *E. dermatitidis* infection noted the ability of this fungus to cause fatal infections of the CNS in otherwise healthy subjects (from Japan, China, Korea, Turkey, Pakistan, with mean age at onset of 30 years [3–75 years]) [72]. Finally, a *CARD9* mutation has been identified in a 37-year-old female Chinese patient with disfiguring subcutaneous phaeohyphomycosis due to *Corynespora cassiicola*, a plant pathogen frequently affecting fruits (e.g. cucumber, tomato) [73] (Table 2). Only four other cases of *C. cassiicola* disease have been reported, mostly in Asia, and all with an onset in late adulthood (over the age of 57 years), and affecting the hands, legs or eyes. The reports to date suggest that mutations of *CARD9* should be assessed in patients presenting with unexplained chronic cutaneous/subcutaneous or invasive disease due to melanin-pigmented fungi, such as *Exophiala* spp., *Phialophora* spp., or *Alternaria* spp. in particular.

CARD9 deficiency and extrapulmonary aspergillosis

Over 500 spp. of *Aspergillus* molds have been identified, but only a few are known to be pathogenic in humans (e.g. *A. fumigatus*, *A. flavus*, *A. niger*, or *A. terreus*). The inhalation of *Aspergillus* spores can cause life-threatening invasive diseases, mostly affecting the lungs, particularly in patients with iatrogenic immunosuppression [74] or PIDs [7]. Two patients with AR *CARD9* deficiency were recently reported to have probable or confirmed extrapulmonary aspergillosis [57]. One of these patients, of mixed European origin, first developed chronic superficial and invasive candidiasis of the liver and mesenteric LN at the age of nine years. Nine years later, he presented cerebral lesions with branching septate hyphae, suggestive of possible cerebral aspergillosis. At 25 years of age, he developed probable intra-abdominal aspergillosis with acute-angle branching septate hyphae on biopsy specimens from hepatic and mesenteric LN lesions. The second patient, of African-American origin, suffered from intraabdominal aspergillosis caused by *A. fumigatus* at the age of eight years, with a large mass surrounding the celiac, mesenteric, and renal arteries, preceded by tinea corporis. A year later, he developed a new adrenal mass caused by *A. fumigatus*. Despite antifungal therapy, surgery, and two stem cell transplants, the patient died three years later, from complications of transplantation. Neither patient developed pulmonary aspergillosis (Table 2). Disseminated extrapulmonary aspergillosis in otherwise healthy patients with no known underlying condition remains rare, with about 300 cases reported as single cases or as small case series. However, the number of cases is probably

underestimated. The most common presentation is as sino-orbital aspergillosis. The disease may, more rarely, affect the brain (through hematogenous dissemination from a distal site, usually the lungs, or direct extension from an adjacent area, e.g. sinus, orbit, ear), internal organs (e.g. liver, LNs, digestive or urinary tract), bones (e.g. vertebral osteomyelitis) or skin [74]. Most cases are reported in Asia (China, Korea, India, Turkey, and Pakistan), the Middle East (Saudi Arabia), or Africa (Sudan). The disease generally has an adult onset (at a mean age of about 36 years, range: 3 months – 74 years). It has a M:F ratio of 1.5, a mortality rate of about 20% and a recurrence rate of about 10%, these two percentages probably being largely underestimated as many patients are lost to follow-up. It generally improves significantly after surgery and antifungal therapy (e.g. amphotericin B, itraconazole, voriconazole). Inherited CARD9 deficiency may underlie some of these cases.

Implications for CARD9 immunity

The main human *CARD9* transcript encodes a 536-amino acid protein with an N-terminal CARD domain and a C-terminal coiled-coil domain (reviewed in [75,76]). CARD9, which is expressed primarily in myeloid cells, functions as an adaptor protein downstream from various receptors of fungal compounds (e.g. β -glucans, α -mannans and glycolipids), including C-type lectins in particular (e.g. Dectin-1; Dectin-2; Dectin-3; MINCLE) [75,76]. CARD9 signaling is, therefore, critical for innate antifungal responses [75,76]. Upon Dectin-1/spleen tyrosine kinase (SYK) activation by fungal β -glucans, CARD9 assembles with B-cell lymphoma/leukemia 10 (BCL10) and mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), to activate NF- κ B, c-Jun N-terminal kinase (JNK) and p38, to stimulate the production of chemokines/cytokines (e.g. TNF, IL-2, or pro-Th17 cytokines) and antigen presentation [77,78]. Patients with AR BCL10 [79] or MALT1 [80–83] deficiency have not been reported to display invasive fungal infections, suggesting that there may be additional molecular pathways involved in protection against such diseases. A new signaling axis, in which CARD9 interacts with Ras protein-specific guanine nucleotide-releasing factor 1 (RASGRF1) to recruit V-Ha-Ras Harvey rat sarcoma viral oncogene homolog (H-RAS) for downstream extracellular signal-regulated kinase (ERK) activation, was recently characterized in mice [84]. The existence of this pathway was subsequently confirmed in humans, as, following zymosan stimulation, CARD9-deficient PBMCs display lower levels of ERK activation and, consequently, impaired granulocyte-monocyte colony-stimulating factor (GM-CSF) production (Figure 2). The success of adjuvant GM-CSF therapy in CARD9-deficient patients with *Candida* CNS disease refractory to antifungal therapy [61,85] also provides support for the biological relevance of this pathway. Furthermore, it has been shown that CARD9-deficient neutrophils have impaired responses to *C. albicans*, in terms of both their capacity to migrate towards foci of fungal infection [56], and in their management of fungi under specific conditions *in vitro* [59,86,87]. Similarly, despite the maintenance of anti-*Aspergillus* effector functions (phagocytosis and killing) or normal neutrophil-intrinsic chemotactic functions, it has been suggested that extrapulmonary aspergillosis results from impaired CARD9-dependant neutrophil recruitment to sites of infection [56,57]. Likewise, the blunted pro-inflammatory responses of patients' PBMCs, as shown for *C. albicans*, *Exophiala* spp., *P. verrucosa*, and *A. fumigatus* [22,52,57,59,61,70,71,85], probably contribute to the impaired neutrophilic response and weaken inherent mechanisms of fungal control. Finally, the impaired or normal

production of IL-17, measured *ex vivo* or *in vitro* after PMA/ionomycin or fungal stimulation, suggests that CARD9 may be important, but not critical, for Th17-cell differentiation, accounting for the incomplete penetrance for CMC in CARD9 deficiency.

Conclusions and future directions

The identification of otherwise healthy patients with CMC and inborn errors of IL-17 immunity has demonstrated not only that CMC is a PID, but also that IL-17A and IL-17F play essential roles in protective immunity against mucocutaneous infections with *C. albicans*. These studies have also suggested that IL-17RA-dependent cytokines are involved in mucocutaneous immunity to *S. aureus*. However, IL-17A and IL-17F seem to be otherwise largely redundant in host defense against other common pathogens, including other bacteria and fungi. They also seem to be redundant for systemic immunity to *C. albicans*, as invasive candidiasis has not been reported in any patient with inborn errors of IL-17 immunity (i.e. with AD IL-17F, AR IL-17RA, IL-17RC, or ACT1 deficiency). The identification of AR CARD9 deficiency has not only revealed that isolated invasive fungal diseases can be caused by a PID, but also the essential role of CARD9 in systemic (as opposed to mucocutaneous) immunity to various fungi — *C. albicans*, dematiaceous fungi, dermatophytes, and *Aspergillus* — and its redundancy for mucocutaneous immunity to these and other fungi. Paradoxically, only one patient displayed invasive infections caused by two or more fungi, suggesting that the expression of CARD9 deficiency may be variable, with incomplete penetrance for each of the mutually exclusive fungal infection phenotypes. It remains unknown whether CARD9 is redundant for systemic immunity against other fungi (e.g. dimorphic fungi, *Cryptococcus* spp.). By contrast to the pathogenesis of CMC in patients with inborn errors of IL-17, much remains unknown about the pathophysiology of invasive fungal diseases in patients with inborn errors of CARD9 immunity. The hematogenous dissemination of *Candida* is symptomatic and life-threatening, but it is unclear why patients with AR CARD9 deficiency can remain asymptomatic for years, or even decades. The factors determining the anatomic location of fungal lesions are also unclear. Finally, the cells and molecules that fail to control fungi in CARD9-deficient patients remain to be identified. In any case, these experiments of Nature demonstrate that fungal diseases may have a genetic determinism, and identification of the disease-causing genes will provide molecular clues to the physiological pathways non-redundant for human antifungal defenses. Ultimately, a comprehensive immunogenetic dissection of “idiopathic” fungal diseases will shed new light on the molecular and cellular mechanisms conferring protective immunity to specific fungi, and pave the way for innovative host-directed therapies based on a better understanding of the underlying pathophysiological mechanisms. Importantly, the clinical implications extend well beyond patients with fungal diseases due to inborn errors of immunity, to patients with fungal diseases due to other causes [16].

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Figure 1. CARD9- and IL-17-mediated immunity in fungal diseases

The recognition of fungal molecules, such as β -glucans, α -mannans, and glycolipids, by C-type lectin receptors (Dectin-1, Dectin-2, Dectin-3, and MINCLE) induces the assembly of a CARD9-BCL10-MALT1 complex, which mediates the activation of NF- κ B and MAPK signaling, leading to the translocation of NF- κ B and AP-1 into the nucleus. Following the recognition of *C. albicans* by Dectin-1, CARD9 also links RASGRF1 to H-RAS and initiates ERK signaling. This pathway ultimately results in the production of pro-inflammatory cytokines, such as IL-6 and GM-CSF, by phagocytes and epithelial cells. Similarly, the binding of IL-17A and IL-17F to the IL-17RA/IL-17RC receptor facilitates the recruitment of ACT1 to the receptor, leading to the activation of NF- κ B, MAPK, and C/EBP signaling, thereby inducing the production of pro-inflammatory cytokines and chemokines (e.g. IL-6, CXCL1), matrix metalloproteinases (e.g. MMP1), and antimicrobial peptides (e.g. β -defensin 3). In T cells, IL-6, IL-23, IL-27, and IFNs (IFN- α , IFN- β , and IFN- γ) bind to their receptors, leading to the recruitment of JAK family kinases to the receptor and the activation of downstream STAT1 and STAT3 signaling. STAT1 activation abolishes the expression of Th17 signature cytokines (e.g. IL-17A, IL-17F), whereas STAT3 activation induces the production of ROR γ T, which cooperates with STAT3 to promote Th17 cell differentiation. IKK, I κ B kinase; NEMO, NF- κ B essential modulator; IFNR, IFN receptor; JAK, Janus kinase; TYK2, tyrosine kinase 2; MMP1, matrix metalloproteinase 1; BD-3, β -defensin 3. Red stars indicate the genetic causes of invasive fungal diseases and of “isolated” chronic mucocutaneous candidiasis.

Figure 2. Fungal taxonomy
The disease-causing fungi identified in CARD9-deficient patients are shown in red.

Table 1

Inborn errors of IL-17 immunity in patients with inherited ‘isolated’ or syndromic CMC

AD, autosomal dominant; AR, autosomal recessive; DN, dominant-negative; LOF, loss-of-function; GOF, gain-of-function; NT, not tested.

Genetic defect	Inheritance	Allele	IL-17A/F signaling	IL-17E signaling	% IL-17 ⁺ CD4 ⁺ T cells	Fungal disease				Bacterial disease		Viral disease	Mycobacterial disease	Ref
						Superficial	Invasive	Superficial	Invasive					
IL-17F deficiency	AD	DN	Impaired	Normal	Normal	Yes	No	No	No	No	No	No	No	
IL-17RC deficiency	AR	LOF	Abolished	Normal	Normal	Yes	No	No	No	No	No	No	No	
IL-17RA deficiency	AR	LOF	Abolished	Abolished	Normal to increased	Yes	No	Yes	No	No	No	No	No	
ACT1 deficiency	AR	LOF	Abolished	Abolished	Normal to increased	Yes	No	Yes	No	No	No	No	No	
CARD9 deficiency	AR	LOF	NT	NT	Low to normal	Yes	Yes	No	No	No	No	No	No	
STAT3 deficiency	AD	DN	NT	NT	Low	Yes	No	Yes	Yes	Yes	Yes	Yes	No	
STAT1 GOF	AD	GOF	NT	NT	Low	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
ROR-γT deficiency	AR	LOF	NT	NT	Absent to low	Yes	No	No	No	No	No	No	Yes	

Table 2
Disease-causing fungi and the associated clinical phenotype described in CARD9-deficient patients

n: number of patients with the corresponding disease.

Candidiasis		Deep dermatophytosis	Phaeohyphomycosis			Extrapulmonary aspergillosis
<i>Candida spp</i>		<i>Trichophyton spp</i>	<i>Phialophora spp</i>	<i>Exophiala spp</i>	<i>Corynespora spp</i>	<i>Aspergillus spp</i>
<i>C. albicans</i>	<i>C. albicans</i> (C, dubliniensis, C. glabrata)	<i>T. violaceum T. rubrum</i>	<i>P. verrucosa</i>	<i>E. dermatitidis</i>	<i>C. cassiicola</i>	<i>A. fumigatus</i>
Superficial (n=20)	Invasive (n=19)	Invasive/extensive (n=21)	Subcutaneous (n=4)	Invasive (n=1)	Subcutaneous (n=1)	Invasive (n=2)
Skin, scalp, nails, oral cavity, esophagus, reproductive tract	CNS (n=16) Osteomyelitis (n=3) Endophthalmitis (n=2) Intra-abdominal (n=1) Colitis (n=2)	Skin/scalp/nails/mucosa (n=21) LN (n=12) Bone (n=1) Viscera (n=1) CNS (n=2)	Subcutaneous, eye	liver and brain	Subcutaneous	Possible CNS, intra- abdominal