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Mendelian traits causing susceptibility to mucocutaneous fungal infections in human subjects

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Activity Objectives

1. To recognize key molecules involved in defense against fungal infections.
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Mucocutaneous candidiasis and dermatophyte infections occur either in isolation or alongside other symptoms in patients with various primary immunodeficiency diseases with diverse genetic defects, which result in impaired IL-17 immunity, IL-22 immunity, or both. In patients with chronic mucocutaneous candidiasis, disease-associated polymorphisms in *DECTIN1* act on the level of fungal recognition, whereas mutations in caspase recruitment domain–containing protein 9 *(CARD9)* disturb the subsequent spleen tyrosine kinase 2–CARD9/BCL10/MALT1–driven signaling cascade, impairing nuclear factor κB–mediated maturation of antigen-presenting cells and priming of naive T cells to differentiate into the T_H17 cell lineage. T_H17-priming cytokines signal through the transcription factor signal transducer and activator of transcription (STAT) 3, which in turn induces the T_H17 lineage–determining transcription factor retinoic acid–related orphan receptor γt. Dominant-negative mutations in *STAT3* result in reduced numbers of T_H17 cells, causing localized candidiasis in patients with hyper-IgE syndrome. In patients with chronic mucocutaneous candidiasis, gain-of-function *STAT1* mutations shift the cellular response toward T_H17 cell–inhibiting cytokines. T_H17 cells secrete IL-17 and IL-22, which are cytokines with potent antifungal properties, including production of antimicrobial peptides and activation and recruitment of neutrophils. Neutrophils mediate microbial killing through phagocytosis, degranulation, and neutrophil extracellular traps. Mutations in *IL17F* and *IL17R* in patients with chronic mucocutaneous candidiasis, as well as neutralizing autoantibodies against IL-17 and IL-22 in patients with autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy, directly impair IL-17 and IL-22 immunity.
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Terms in boldface and italics are defined in the glossary on page 295.

Abbreviations used

AD: Autosomal dominant
AIRE: Autoimmune regulator
APECED: Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy
AR: Autosomal recessive
BCL10: B-cell lymphoma/leukemia 10
CARD9: Caspase recruitment domain–containing protein 9
CMC: Chronic mucocutaneous candidiasis
DC: Dendritic cell
DC-SIGN: Dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin
DOCK8: Dedicator of cytokinesis 8
HIES: Hyper-IgE syndromes
ITAM: Immunoreceptor tyrosine-based activation motif
MALT1: Mucosa-associated lymphoid tissue lymphoma translocation protein 1
MAP: Mitogen-activated protein
MINCLE: Macrophage-inducible C-type lectin
mTEC: Medullary thymic epithelial cell
MyD88: Myeloid differentiation primary response gene 88
NF-κB: Nuclear factor κB
NLRP3: NLR family, pyrin domain containing 3
NOD: Nucleotide-binding oligomerization domain
PID: Primary immunodeficiency
PRR: Pattern-recognition receptor
ROR: Retinoic acid–related orphan receptor
SCID: Severe combined immunodeficiency
STAT: Signal transducer and activator of transcription
Syk: Spleen tyrosine kinase
TLR: Toll-like receptor

Although the gut is the organ primarily colonized with *Candida* species, fungal infections in human subjects mainly occur on mucocutaneous surfaces, such as the skin, nails, oral cavity, and genitals. In addition to these, the lungs can be involved in an immune-compromised host. In the latter patients fungal infections can become systemic. If they do, systemic fungal infections are associated with high mortality. Risk factors for mucocutaneous candidiasis include the intake of broad-spectrum antibiotics, which alter the balance of the microbial flora and allow for the overgrowth of fungi; diabetes mellitus because of high glucose levels in the tissues and a subsequent dysfunction of immune cells; a weak immune system, such as found at very young or old age; chemotherapy or steroid treatment; AIDS; and inborn errors of immunity (Table I).[1,2] One such inborn immunodeficiency is named chronic mucocutaneous candidiasis (CMC), but fungal infections (mainly with *Candida* species) also occur in patients with other selected primary immunodeficiencies (PIDs). In this review we will discuss the gene defects of these PIDs and how specific genes contribute to antifungal defense.

CANDIDA SPECIES INFECTIONS

Candida species, primarily *Candida albicans*, which are commensal yeasts in the orogastrointestinal flora of healthy subjects, are the most prevalent opportunistic pathogens in patients with PIDs. Some *Candida* species, including *Candida albicans*, can either grow as unicellular yeast or as branching filamentous hyphae, and ***germination*** and hyphae formation are critical for tissue invasion and fungal pathogenicity on epithelial surfaces.[3,4]

Oral *Candida* species infection (thrush) presents as thick white-to-yellowish ***pseudomembranes*** consisting of fungi, debris, and inflammatory cells, with the underlying tissue massively inflamed.[5] Oral candidiasis is very common in patients with CMC[6] and other PIDs, such as hyper-IgE syndromes (HIES)[7,8] and autoimmune polyendocrinopathy–candidiasis–***ectodermal dystrophy*** (APECED),[9] as well as in HIV-infected patients.[10]

In patients with chronic diffuse candidiasis, the infection affects the mucosa and can spread to the skin of the scalp, upper body, and extremities, as well as to the perianal and perineal area and nails.[5,11] *Candida* species granulomas can form in patients

GLOSSARY

ANTIMICROBIAL PEPTIDES (AMPS): Components of the innate immune system that are capable of inserting into bacterial phospholipids to slow microbial growth. AMP levels are decreased in the skin of patients with atopic dermatitis.

CASPASES: Enzymes that are cysteinyl proteases that cleave after specific aspartyl residues. Caspases are involved in programmed cell death. A small number of autoimmune lymphoproliferative syndrome cases are caused by mutations in caspase-10.

ECTODERMAL DYSTROPHY: A series of defects to ectodermic structures, including pitted nails, enamel hypoplasia, and keratopathy. The ectoderm is the outermost germ layer in a developing embryo.

GERMINATION: To develop or grow; in botany the process of seeds or spores beginning to grow new tissue.

IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIF (ITAM): Conserved tyrosine-containing peptide sequences that, once phosphorylated, serve as "docking sites" for additional signaling molecules.

NATURAL REGULATORY T CELLS: $CD4^+$ regulatory T cells that develop in the thymus and constitutively express forkhead box protein 3 (Foxp3) and CD25.

NEUTROPENIA: A low absolute neutrophil count, generally accepted as less than 1500 cells/μL.

NLR FAMILY, PYRIN DOMAIN CONTAINING 3 (NLRP3) INFLAMMASOME: Also referred to as the NALP3 inflammasome, an enzyme complex that functions to activate the potent proinflammatory molecules IL-1, IL-18, and IL-33. Alum is a vaccine adjuvant that is taken up by phagocytic cells, where it activates NALP3. Mutations in NALP3 result in the cryopyrin-associated periodic syndromes.

NONSENSE MUTATION: Genetic information that does not code for any amino acid but is the stop codon causing termination of the molecular chain in protein synthesis.

PERLÈCHE: A superficial inflammatory condition of the angles of the mouth, often with fissuring that is caused especially by infection or avitaminosis.

PSEUDOMEMBRANE: A fibrinous deposit with enmeshed necrotic cells. It is also known as a false membrane.

REACTIVE OXYGEN SPECIES: Substances (eg, hydrogen peroxide) typically generated at a low frequency during oxidative phosphorylation in the mitochondria, as well as in a variety of other cellular reactions. Reactive oxygen species are capable of exerting cellular damage by reacting with intracellular constituents, such as DNA and membrane lipids.

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TABLE I. Factors enhancing susceptibility to *Candida* species

Broad-spectrum antibiotics
Steroid treatment
Chemotherapy
Diabetes mellitus
Dentures
Weak immune system (as found at very young or old age)
HIV infection/AIDS
Certain PIDs

with localized mucocutaneous candidiasis.[1] Granulomas are hyperkeratotic cutaneous lesions that form thick crusts and are infiltrated by inflammatory cells.[5] Those patients can have life-impairing disabilities.

OTHER FUNGAL INFECTIONS

Dermatophyte infections are cutaneous infections with *Microsporum*, *Epidermophyton*, and *Trichophyton* species that extend into the epidermis of the skin and also include invasive hair and nail infections. Dermatophytoses can be severe, resulting in disfigurements.[12,13] Tinea infections (commonly called ringworm) are caused by *Trichophyton* species and can affect the skin of the body (tinea corporis), feet (tinea pedis), groin area (tinea cruris), and scalp (tinea capitis). Tinea versicolor is caused by *Malassezia* species and presents as hypopigmented spots on the skin.[14] Other opportunistic fungal infections include aspergillosis and cryptococcosis. *Aspergillus fumigatus* is the main fungus causing pathology, ranging from allergic bronchopulmonary aspergillosis, which is characterized by an exaggerated immune response to the fungus, allergic fungal sinusitis, and pulmonary aspergillomas, to invasive aspergillosis, a leading cause of death in patients undergoing hematopoietic stem cell transplantation and patients with acute leukemia.[15] Infections with *Cryptococcus neoformans* affect mainly immunocompromised patients.[16,17] In addition to cutaneous and pulmonary cryptococcosis, invasive infections of the central nervous system can occur, leading to cryptococcal meningitis.[18] Invasive central nervous system infections, however, can also be caused by *Candida* species and *Histoplasma capsulatum* (Table II).[12,19]

Interestingly, other fungal infections, such as histoplasmosis, blastomycosis, and coccidiomycosis (Table II), have not been observed in patients with the genetic diseases discussed below.

IMMUNITY TO *CANDIDA* SPECIES

The first line of defense against invading microbes is provided by the skin and mucosa, which, in addition to serving as physical barriers, contain ***antimicrobial peptides***, such as β-defensins.[20] Microbial growth on body surfaces is also controlled by the local physiologic flora. The second line of defense against *Candida* species is composed of an interplay between the innate and adaptive immune systems. Localized candidiasis, such as CMC, is the result of impaired cellular immunity and can be found in patients with T-cell defects (HIV/AIDS, severe combined immunodeficiency [SCID], and dedicator of cytokinesis 8 [DOCK8] deficiency).[21-23] A defective innate immune system, such as seen in patients with congenital ***neutropenias*** or neutropenia after chemotherapy, is more severe and predisposes to systemic candidiasis.[11,24]

C albicans has a cell wall rich in components that are absent in human cells but are essential for the structure of the fungal cell, such as chitin, the major structural polymer; β-glucans; mannans; and mannoproteins.[25] These pathogen-associated molecular patterns are recognized by pattern-recognition receptors (PRRs) of the innate host defense system, in particular dendritic cells (DCs) and macrophages.[26] The transmembrane PRRs Toll-like receptors (TLRs) and C-type lectin receptors are both critically involved in antifungal host defense,[27,28] whereas cytosolic receptors, such as retinoic acid–inducible gene I and nucleotide-binding oligomerization domain (NOD) proteins, sense intracellular bacteria and viruses (Fig 1).[29] Pathways meet at central signaling adaptors to allow for cross-talk and synergistic interactions to fine tune the immune response.

Different components of the *C albicans* cell wall are recognized by different receptors. Recognition of mannans in the outer portion of the cell wall is mediated by TLR4, the mannose receptor, dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN), and dectin-2, whereas dectin-1 and macrophage-inducible C-type lectin (MINCLE) bind to β-glucans in the inner portion of the fungal cell wall (Fig 1).[20,30-34] It appears that C-type lectin receptors, such as dectin-1 and dectin-2, DC-SIGN, and MINCLE are the main receptors in the recognition of *C albicans*. They signal through a complex containing spleen tyrosine kinase (Syk) and ***caspase*** recruitment domain–containing protein 9 (CARD9), whereas TLR4 and the phospholipomannan-binding receptor TLR2 signal through the adaptor proteins myeloid differentiation primary response gene 88 (MyD88) and Mal to activate nuclear factor κB (NF-κB) and mitogen-activated protein (MAP) kinase.[28] Dectin-1 can amplify proinflammatory responses by TLR2.[35]

In mice MyD88 is required for host defense against *C albicans* and *A fumigatus*.[28,36] The role of TLRs is dependent on fungal species and morphotypes. TLR4 is involved in protection against disseminated *C albicans* infection, as well as cytokine responses to *A fumigatus* conidia. TLR2 recognizes *C albicans* but not *A fumigatus* hyphae. In response to *C albicans*, TLR2 can also induce immunomodulatory effects through the activation of regulatory T cells.[28]

Dectin-1 and dectin-2: Recognition of *C albicans* and induction of signaling

Dectin-1 is thought to play a pivotal role in mucosal antifungal defense in human subjects and mice.[37-40] Being a PRR, dectin-1 signaling activates innate immune responses, such as phagocytosis and production of ***reactive oxygen species*** and inflammatory cytokines and chemokines.[41,42] Furthermore, dectin-1 signaling shapes adaptive immune responses.

Dectin-1 is expressed on macrophages, DCs, and neutrophils, where it forms a "phagocytic synapse" when binding to particulate β-glucans on direct microbial contact. Only activation through the phagocytic synapse leads to dectin-1 signaling[43] because responses like phagocytosis and oxidative burst are only useful in pathogen clearance when the phagocyte comes into direct contact with the microbe. In contrast, inflammatory responses by TLRs can be activated by soluble components released from microbes further away.[43]

TABLE II. Human pathogenic fungi

Disease	Pathogenic fungus	Symptoms
Candidiasis	*Candida* species, mainly *Candida albicans*	Thrush; infection of genitals, nails, mucosa, scalp, skin of upper body and extremities; granuloma; invasive central nervous system infection
Aspergillosis	*Aspergillus* species, mainly *Aspergillus fumigatus*	Pulmonary aspergillosis; invasive infection; allergic bronchopulmonary aspergillosis; allergic sinusitis
Cryptococcosis	*Cryptococcus* species, mainly *Cryptococcus neoformans*	Cutaneous and pulmonary infection; cryptococcal meningitis
Dermatophytosis	Dermatophytes: *Microsporum* species *Epidermophyton* species *Trichophyton* species	Cutaneous infections extending into epidermis; invasive hair and nail infection
Histoplasmosis	*Histoplasma capsulatum*	Pulmonary and cutaneous histoplasmosis; granuloma; disseminated infection
Blastomycosis	*Blastomyces dermatitidis*	Pulmonary and cutaneous infection
Coccidiomycosis	*Coccidioides immitis*	Pulmonary coccidiomycosis; cutaneous infection; disseminated infection; granulomatous meningitis

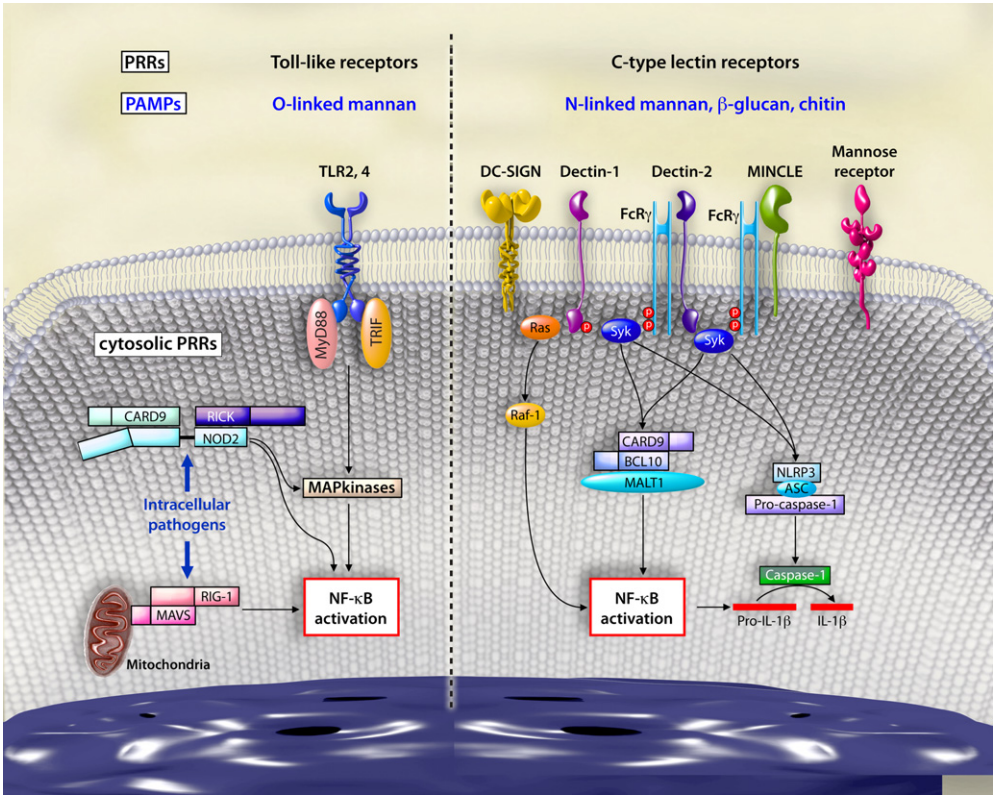

FIG 1. PRRs recognizing different fungal cell-wall components. Activation of TLRs by O-linked mannans results in MyD88- and TRIF-mediated activation of NF-κB. C-type lectin receptors are activated by N-linked mannans, β-glucan, and chitin. Dectin-1, dectin-2, and MINCLE recruit Syk and signal through a CARD9/BCL10/MALT1-containing complex for the activation of NF-κB and through the NLRP3 inflammasome for caspase-1–mediated cleavage of pro–IL-1β into IL-1β. Intracellular pathogens are recognized by cytosolic PRRs, such as NOD2 and retinoic acid–inducible gene I *(RIG-1). ASC,* Apoptosis-associated speck-like protein containing a CARD; *MAVS,* mitochondrial antiviral signaling; *PAMPs,* pathogen-associated molecular patterns; *PRRs,* pattern recognition receptors; *Ras,* rat sarcoma; *RICK,* RIP-like interacting CLARP kinase; *TRIF,* Toll-receptor-associated activator of interferon.

The cytoplasmic tail of dectin-1 harbors a "hemITAM" that resembles the ***immunoreceptor tyrosine-based activation motif*** (ITAM) found in many immune receptors. On ligand binding and exclusion of phosphatases from the synapse, the hemITAM is tyrosine phosphorylated by Src, followed by recruitment of Syk through phosphotyrosine/Src homology 2 domain interactions. Activation of Syk leads to the activation of gene transcription and production of proinflammatory cytokines and chemokines in a CARD9-dependent manner, whereas signals for phagocytosis are independent of CARD9 (Fig 2).[44]

Dectin-2, which is thought to be even more important in the defense against *Candida* species,[45] is expressed on macrophages and DCs, binds fungal α-mannans, recruits the ITAM-containing cell-surface receptor FcRγ, and, similarly to dectin-1, signals through the ITAM/Syk/CARD9 signaling axis to induce antifungal immunity (Fig 2).[45-47]

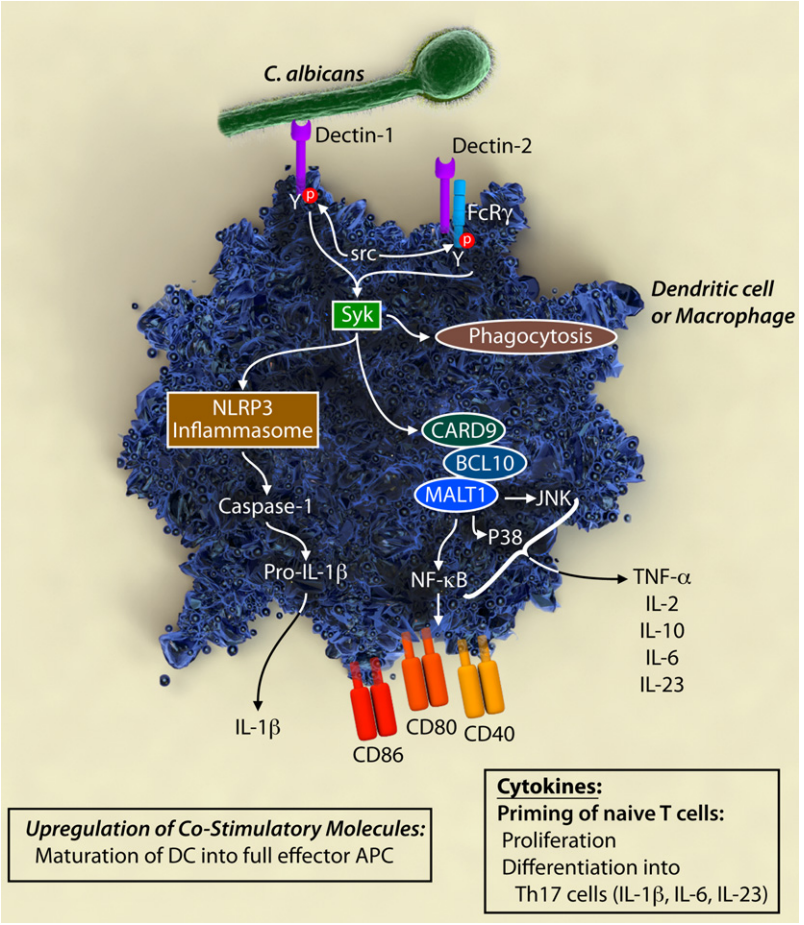

FIG 2. Signaling pathways downstream of dectin-1 and dectin-2 on binding of *Candida albicans*. Binding of *C albicans* to dectin-1 and dectin-2 leads to Src-mediated tyrosine phosphorylation of the hemITAM of dectin-1 or the ITAM of the dectin-2–associated FcR γ chain. Subsequent recruitment of Syk drives phagocytosis, formation of a CARD9/BCL10/MALT1 signaling complex, and activation of the NLRP3 inflammasome. The CARD9-containing complex leads to activation of NF-κB and the MAP kinases p38 and c-Jun N-terminal kinase (JNK), resulting in upregulation of costimulatory molecules and production of T_H17-priming cytokines. Activation of caspase-1 in the context of the NLRP3 inflammasome results in cleavage of pro–IL-1β and secretion of active IL-1β, which also contributes to differentiation of naive T cells into T_H17 cells.

Dectin-2 is also a PRR for *A fumigatus* on DCs, and it signals through FcRγ to generate cysteinyl leukotrienes in the allergic response to the fungus.[48]

CARD9: A central antimicrobial adaptor molecule

The CARD adaptor protein CARD9 is highly expressed in macrophages and myeloid DCs, in which it transmits signals emerging from various microbe-sensing receptors to core transcription factors. Pathways initiated by ITAM-containing or ITAM-coupled receptors on myeloid cells and pathways initiated by TLRs and NOD2 converge on CARD9.

CARD9 is downstream of all myeloid receptors that either contain an ITAM motif or couple to ITAM-containing molecules and recruit and activate tyrosine kinases, such as Syk.[44] After Syk activation, CARD9 forms a signaling complex with B-cell lymphoma/leukemia 10 (BCL10) and variably mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), leading to activation of NF-κB. This is analogous to the pathway in lymphocytes, in which antigen receptors, through Syk, engage a signaling complex comprising the CARD domain–containing adaptor CARD11 and BCL10 to direct activation of NF-κB.[44]

NOD2, the cytoplasmic sensor of intracellular bacteria, binds to bacterial peptidoglycans and forms a complex with CARD9 and the serine/threonine kinase RICK. In this complex CARD9 is crucial for the activation of MAP kinases, whereas RICK transmits the signal to NF-κB (Fig 1).[49]

In response to viruses, CARD9 plays a role downstream of the intracellular receptors TLR3 and TLR7, which recognize viral double-stranded RNA. The role of CARD9 directly downstream of transmembrane TLRs is not clear. However, when CARD9 is activated through an ITAM-containing receptor, it plays a role in the enhancement of TLR signaling after various stimuli.[44]

In antifungal immunity engagement of dectin-1 on macrophages and DCs through binding of *C albicans*, β-glucan, or zymosan (a fungal cell-wall preparation enriched in β1-3 and β1-6 linked β-glucans) leads to a tyrosine phosphorylation cascade involving Src and Syk and formation of a complex containing CARD9, BCL10, and MALT1.[50] This complex drives activation of NF-κB and the MAP kinases p38 and c-Jun N-terminal kinase, which results in the production of the cytokines TNF-α, IL-2, IL-6, IL-10, and IL-23, as well as upregulation of the costimulatory molecules CD40, CD80, and CD86 (Fig 2).[44,51-54] Furthermore,

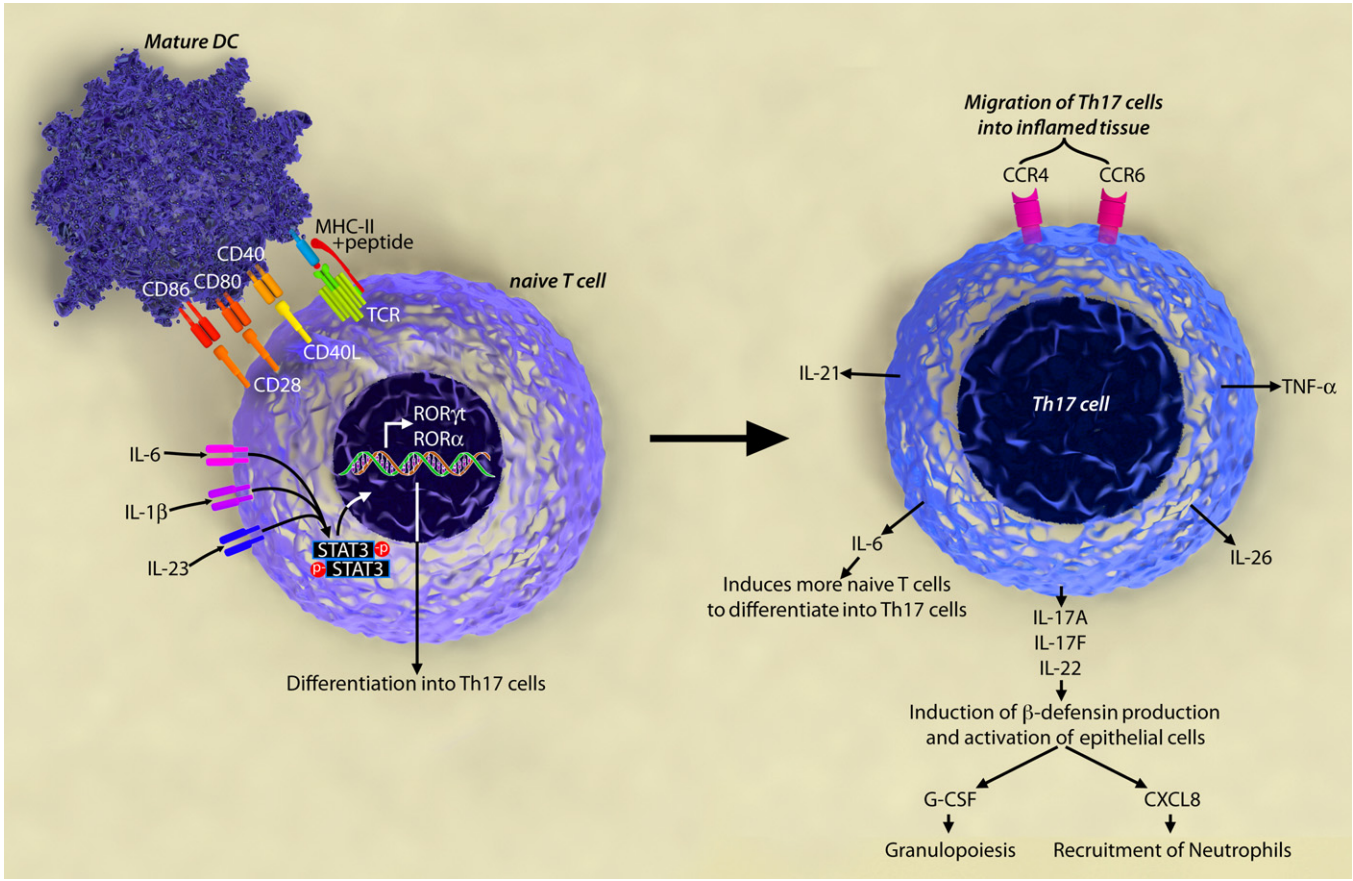

FIG 3. Development and effector function of T_H17 cells. The cytokines IL-6, IL-1β, and IL-23 drive the differentiation of naive T cells, which receive antigen-specific and costimulatory signals provided by mature antigen-presenting cells, into T_H17 cells. These cytokines signal through STAT3, which in its phosphorylated and dimerized form translocates into the nucleus to induce transcription of the T_H17 lineage–determining transcription factors RORγt and RORα. T_H17 cells upregulate tissue-homing chemokine receptors, such as CCR4 and CCR6, and produce a distinct set of cytokines. This includes the signature cytokines IL-17A, IL-17F, and IL-22. The main outcome is activation of epithelial cells; production of antimicrobial peptides, such as β-defensins; granulopoiesis; and recruitment of neutrophils to the site of infection.

Syk leads to the activation of the caspase-1–containing ***NLR family, pyrin domain containing 3 (NLRP3) inflammasome***, which processes pro–IL-1β that had been induced by NF-κB into bioactive IL-1β (Fig 1).[52-54]

All these cytokines in combination with maturation of DCs into full effector antigen-presenting cells can prime naive T cells to proliferate and differentiate into $CD4^+$ T_H cells of the T_H1 and T_H17 lineages. Whereas T_H1 cells secrete IFN-γ and have an important proinflammatory role, T_H17 cells produce IL-17 and IL-22, which have an important role in neutrophil recruitment and antifungal immunity in general (see below).

Therefore an important outcome from *C albicans*–induced CARD9 signaling seems to be the coupling of innate to adaptive immunity, resulting in the generation of *C albicans*–specific T_H17 cell responses.

Differentiation into T_H17 cells: Requirement of the transcription factor signal transducer and activator of transcription 3

In 2005, IL-17–producing $CD4^+$ T cells were identified as a separate T_H cell lineage, shaping the immune response through the secretion of a distinct set of cytokines.[55,56]

Naive $CD4^+$ T cells that receive low-strength activation signals trough their T-cell receptors[57] differentiate into T_H17 cells on stimulation with IL-1β, IL-6, IL-23, and possibly low concentrations of TGF-β.[58-60] This leads to the activation of the transcription factor signal transducer and activator of transcription (STAT) 3 and, subsequently, to induction of the lineage-determining transcription factors retinoic acid–related orphan receptor (ROR) γt (in human subjects referred to as Rorc2) and RORα (Fig 3).[61,62] Once activated, IL-23 is required for the maintenance of T_H17 cells.[63]

The development of T_H17 cells is inhibited by IFN-β and IL-4, the cytokines produced by T_H1 and T_H2 cells, respectively.[55,56]

T_H17 cells and their cytokines: Stars among antifungal immune cells

T_H17 cells play a role in autoimmunity, as well as in host defense to various extracellular pathogens, including fungi, bacteria, and some parasites.[64,65]

T_H17 cells' signature cytokines are IL-17A and IL-17F. IL-17 homodimers and heterodimers drive the transcription of innate target genes through activation of NF-κB. The major outcome is activation and recruitment of neutrophils and induction of antimicrobial

peptides, such as β-defensins.[66,67] Whereas the latter is a direct result of IL-17–mediated gene expression, the recruitment and expansion of neutrophils occurs through activation of epithelial cells at sites of infection, in particular through secretion of granulocyte colony-stimulating factor, which promotes granulopoiesis, and neutrophil chemoattractant chemokines, such as CXCL8 (IL-8; Fig 3).[58,68,69]

T_H17 cells additionally produce the proinflammatory cytokines TNF-α and IL-6, as well as IL-21, IL-22, and IL-26. IL-6 leads to the differentiation of more naive $CD4^+$ T cells into T_H17 cells,[70] whereas IL-22 enhances the expression of antimicrobial peptides in cooperation with IL-17.[71] The role of IL-21 in human T_H17 cell biology is not quite clear, but it might function through upregulation of IL-17 production and downregulation of regulatory T-cell function.[72,73]

Furthermore, activation of T_H17 cells induces the upregulation of the chemokine receptors CCR4 and CCR6, which drive the migration of T_H17 cells to inflamed skin and mucosa (Fig 3).[74,75]

Thus T_H17 cells' main role in antifungal immunity is at sites of infection in the skin and mucosa through the release of proinflammatory factors, recruitment of neutrophils, and production of antimicrobial peptides.

Neutrophils: The final killers

Neutrophils have 3 main mechanisms to directly kill invading microbes: phagocytosis, degranulation and activation of the oxidative burst, and neutrophil extracellular traps.[76-78]

Microbes are taken up by phagocytosis and are then destroyed by reactive oxygen species with antimicrobial potential, which are produced in a process called oxidative or respiratory burst. Through degranulation, neutrophils release proteins with lytic and antimicrobial function, such as cathepsins, defensins, myeloperoxidase, and bactericidal/permeability-increasing protein. Finally, neutrophils can release so-called neutrophil extracellular traps, which act as a mesh to trap and kill microorganisms independently of phagocytic uptake. The traps consist of a web of DNA and histones and contain granule-derived proteins with antimicrobial activity.[76]

PIDS WITH SUSCEPTIBILITY TO FUNGAL INFECTIONS

With T_H17 cells playing a central role in the defense against fungi in human subjects, it is not surprising to find defects in T_H17 immunity in inborn errors of the human immune system with susceptibility to fungal infections.

Because T_H17 cells function primarily in the skin and mucosa, patients with diseases with profound T-cell defects (ie, CD4 T-cell defects), such as HIV/AIDS, SCID, DiGeorge syndrome, and others, tend to have oral and mucosal candidiasis.[79,80] In patients with these defects, neutrophils are functional and can prevent invasive fungal infections, but because of the lack of T_H17 cell–produced cytokines, trafficking to sites of infection is impaired, leading to local candidiasis. Systemic fungal infections occur in neutropenias or in diseases with neutrophil dysfunction, such as chronic granulomatous disease with defective oxidative burst.[81,82]

Defects leading to diminished T_H17 responses in patients with PIDs with fungal susceptibility occur on several levels in the pathway leading from fungal recognition to differentiation into T_H17 cells exerting their effector function.

Mutations in *DECTIN1* and *CARD9* act on the level of fungal recognition and subsequent signaling.[37,83] Dominant-negative mutations in *STAT3* inhibit the differentiation into T_H17 cells.[84-86] Gain-of-function mutations in *STAT1* shift the immune response toward STAT1-dependent cytokines that inhibit the generation of T_H17 cells.[87,88] Mutations in *IL17F* and *IL17RA*, as well as autoantibodies against IL-17, IL-22, or both, inhibit the effector function of T_H17 cells.[89-91] Finally, at the end of the antifungal pathway, neutrophil defects, such as those seen in patients with severe congenital neutropenia, lead to CMC.[92,93]

Dectin-1 deficiency

Dectin-1 deficiency is a mild immunodeficiency that was described in a Dutch family with 3 affected sisters presenting with recurrent vulvovaginal candidiasis, chronic dermatophyte infection of the nail beds (onychomycosis), or both.[37] A homozygous ***nonsense mutation*** (Y238X) in *DECTIN1* resulted in the loss of a cysteine bond, which was predicted to disrupt correct protein folding. As a consequence, cell-surface expression of the mutated receptor and the capability to bind β-glucan or *C albicans* was lost. Both monocytes and macrophages from patients showed poor *in vitro* production of IL-6, IL-17, and TNF-α on stimulation with β-glucan, *C albicans* yeast, or *C albicans* hyphae. Furthermore, the amplifying effect of dectin-1 on TLR2 signaling in respect to cytokine production was lost in patients' cells. In contrast, phagocytosis and killing of *C albicans* were normal, suggesting a redundant role of dectin-1 in these processes and explaining why no invasive fungal infections occurred in patients with dectin-1 deficiency.

However, the heterozygous parents also had onychomycosis, albeit with a much later onset (40 and 55 years compared with 10 and 12 years); in addition, the father had only transient candidiasis with full recovery. Their cells had intermediate expression of dectin-1 and intermediate IL-6 production after β-glucan or yeast stimulation. Patients with a heterozygous *DECTIN1* mutation show a heavier colonization with *Candida* species than unaffected subjects. Therefore the Y238X polymorphism might be more of a risk factor for a mild form of mucocutaneous candidiasis rather than causing full-blown CMC. In line with this is the finding that the heterozygous Y238X polymorphism is also found in healthy subjects with a heterozygosity frequency of up to 40% in some populations. Healthy subjects with a homozygous Y238X polymorphism have also been discovered (personal communication).

Thus there is no definite answer to the exact contribution of dectin-1 in the pathogenesis of CMC, especially in view of the important role that dectin-2 is playing in antifungal immunity.

Even in mice the contribution of dectin-1 to the susceptibility to *C albicans* is not clear. Whereas Taylor et al[40] found lower survival, increased fungal burdens, and enhanced fungal dissemination on intravenous infection of dectin-1 knockout mice with *C albicans*, the dectin-1 knockout mice of Saijo et al[39] showed no enhanced susceptibility to intravenously administered *C albicans*; however, they did show an impaired response to *Pneumocystis carinii*.[39]

CARD9 deficiency

A homozygous loss-of-function nonsense mutation in *CARD9* was reported in 4 patients from a large consanguineous family

from Iran who had recurrent oral candidiasis, vaginal candidiasis, or both and ***perlèche*** (angular cheilitis), as well as tinea corporis and dermatophytosis.[83] The Q295X mutation resulted in a premature stop codon in the coiled-coil domain of *CARD9* and in the lack of CARD9 expression. Patients had low numbers of IL-17–producing T cells. Experiments using murine CARD9-deficient myeloid cells showed that the Q295X mutation impairs dectin-1 signaling because the production of TNF-α in response to the selective dectin-1 agonist curdlan was impaired but restored by using CARD9-reconstituted myeloid cells.[83]

Recently (unpublished data), we identified further families from Algeria with a homozygous nonsense mutation (Q289X) in the coiled-coil domain of *CARD9*. The hallmark clinical features in these families were dermatophyte infections with *Trichophyton violaceum* and *Trichophyton rubrum* on the skin, scalp, nails, and lymph nodes.

Thus it appears that CARD9 has a crucial role in human antifungal defense downstream of dectin-1, probably through the CARD9/BCL10/MALT-1 signaling complex, leading to a defect in the generation of T_H17 immunity. However, CARD9 seems to have a redundant role in the response to intracellular bacteria and viruses through the NOD/RICK complex because CARD9-deficient patients have no increased susceptibility to bacterial or viral infections. Possibly other members of the CARD family, such as CARD6 or CARD11, might substitute for CARD9 in these immune responses. These findings are supported by the murine model because *Card9* knockout mice have an increased susceptibility with reduced survival to infection with *C albicans* but not *Staphylococcus aureus*, and *Card9*-deficient DCs have severe defects in fungal-induced cytokine production.[50]

HIES: STAT3 and DOCK8 deficiency

Susceptibility to fungal infections (mainly *Candida* species but also *Aspergillus* and *Cryptococcus* species) is part of autosomal dominant (AD) HIES, a multisystem disorder, which is characterized by recurrent *S aureus* infections of the skin and pulmonary tract, high serum levels of IgE, eosinophilia, eczema, and skeletal and dental abnormalities in addition to oral and mucocutaneous candidiasis.[7,8]

Heterozygous dominant-negative *STAT3* mutations account for the majority of patients with AD-HIES.[84,94-96] STAT3 is downstream of T_H17-inducing cytokines, such as IL-6 and IL-23, and is essential for induction of the T_H17 lineage–determining transcription factor RORγt. Thus STAT3-deficient patients show markedly decreased RORγt expression and defective T_H17 cell differentiation.[84-86,97] In addition, T-cell supernatants from patients with HIES were unable to induce β-defensins, probably because of defects in IL-22 generation and signaling, both of which are dependent on STAT3.[85,98] Therefore the susceptibility to *Candida* species in patients with HIES with *STAT3* mutations is probably due to the failure of dominant-negative STAT3 to mount a T_H17 cell response, which impairs β-defensin production and neutrophil trafficking to sites of infection in the skin and mucosa. Furthermore, oral candidiasis is encouraged by reduced antifungal activity in the saliva of STAT3-deficient patients with reduced expression of antimicrobial effectors, such as β-defensin 2 and histatins.[99]

Candidiasis is also a feature of autosomal recessive (AR) HIES.[100] Some patients with this form of the disease have mutations in *DOCK8* and were shown to have defective T_H17 cell differentiation as well, although the underlying mechanism appears to be different from that of STAT3 deficiency.[101-103] Al Khatib et al[101] suggest that unlike in patients with STAT3 deficiency, the induction of RORγt expression in naive T cells was still intact. However, in PBMCs with less naive and more memory T cells, RORγt expression was severely decreased and the production of IL-17 was strongly reduced. Therefore the defect in the T_H17 differentiation pathway seems to be further downstream, affecting distal steps in T_H17 cell differentiation, long-term persistence, or both.

Gain-of-function *STAT1* mutations

Heterozygous missense mutations in the coiled-coil domain of *STAT1* were first discovered by van de Veerdonk et al.[88] Liu et al[87] subsequently elucidated the molecular pathophysiology behind this mutation. Unlike loss-of-function mutations in the Src homology 2– or DNA-binding domain of *STAT1*, which lead to clinical pictures of increased susceptibility to mycobacteria and viruses, the mutations in the coiled-coil domain are associated with susceptibility to mucocutaneous fungal infections. Liu et al showed that these mutations have a gain-of-function effect by reducing the dephosphorylation of activated STAT1, leading to accumulation of phosphorylated STAT1 in the nucleus. The dominance of activated STAT1 shifts the immune response toward STAT1-dependent IL-17 inhibitors and away from STAT3-mediated induction of T_H17 cell generation, which might explain the clinical picture of CMC.

In the first publication, van de Veerdonk et al[88] reported on 14 patients from 5 families of Dutch and British decent who had heterozygous *STAT1* coiled-coil domain missense mutations. Patients presented with AD CMC, severe oropharyngeal chronic candidiasis, and severe dermatophytosis, together with autoimmune phenomena, such as hypothyroidism and autoimmune hepatitis. One patient also had squamous cell carcinoma.

Patients' PBMCs showed poor production of T_H1 and T_H17 cytokines (IFN-γ and IL-17/IL-22, respectively) in response to *C albicans*. In contrast, monocyte-dependent cytokine responses were normal, as was the IFN-γ signaling pathway, which is impaired in loss-of-function *STAT1* mutations. Intact STAT1-mediated IFN-γ responses might explain the normal susceptibility to mycobacteria and viruses.

In the report by Liu et al,[87] 12 different heterozygous *STAT1* coiled-coil domain missense mutations were found in 47 patients from 20 families with CMC; some patients had additionally thyroid autoimmunity, and 1 had a squamous cell carcinoma. Analysis of 1 mutant *STAT1* allele (R274Q) showed stronger cellular responses to key STAT1-activating cytokines, such as IFN-α, IFN-γ, or IL-27, as measured by levels of STAT1 phosphorylation and STAT1-dependent γ-activated sequence transcriptional activity. The higher levels of STAT1 tyrosine 701 phosphorylation in response to IFN-γ were due to impaired nuclear dephosphorylation.

Stimulation of patients' EBV-B cells with IL-6 and IL-21, cytokines that in the healthy state primarily activate STAT3, led to increased STAT1 phosphorylation alongside normal STAT3 activation, suggesting a shift of the immune response away from IL-6/IL-21–induced STAT3-mediated T_H17 cell generation.

Therefore the dominance of gain-of-function STAT1 responses acts in 2 ways with regard to impaired generation of T_H17 cells: first, responses to cytokines that antagonize the development of T_H17 cells, such as IL-27 and IFN-α, are increased, and second, responses to cytokines that normally promote T_H17

differentiation through the activation of STAT3 are shifted toward STAT1. As expected from less activation and more inhibition of the T_H17 differentiation pathway, patients with gain-of-function *STAT1* mutations have reduced proportions of circulating T_H17 cells with poor production of IL-17 and IL-22 *ex vivo*. Interestingly, the patients with the most severe clinical phenotype had the greatest reduction in T_H17 cytokine levels, suggesting that this is indeed the underlying cause for the localized fungal susceptibility found in patients with CMC.

IL-17F and IL-17RA deficiency

In 2008, Eyerich et al[104] studied a group of patients with isolated CMC in whom no other infectious or autoimmune manifestations occurred and showed a smaller proportion of IL-17–producing T cells and low levels of IL-17. These results suggested a protective role of IL-17 in anti-*Candida* species host defense, which was confirmed in 2011 by Puel et al,[91] who reported on 2 genetic defects leading to CMC. One is the AD deficiency of IL-17F, and the other is the AR deficiency of the receptor for IL-17 (IL-17RA).

AR IL-17RA deficiency was found in a child from consanguineous parents of Moroccan origin. The patient presented with neonatal *C albicans* skin infection, followed later by *S aureus*–induced dermatitis. He displayed a homozygous nonsense mutation (Q284X) with a premature stop codon in the extracellular domain of *IL-17RA,* leading to absent surface expression of the receptor on fibroblasts, PBMCs, monocytes, and $CD4^+$ T cells, as well as $CD8^+$ T cells. Even though the proportion of IL-17A– and IL-22–producing T cells was normal, IL-17 immunity was defective because of completely abolished responses to IL-17 homodimers and heterodimers in fibroblasts and PBMCs with regard to IL-6 induction.

AD IL-17F deficiency was found in a multiplex family from Argentina with 5 members with symptoms of CMC. A heterozygous missense mutation (S65L) with incomplete clinical penetrance was found in the *IL17F* gene. The mutated amino acid is conserved across mammalian species and lies in a region of the protein that is involved in cytokine-receptor interactions. The mutation was reported to have a hypomorphic dominant-negative effect by impairing receptor binding of IL-17F homodimers and IL-17F/A heterodimers and reducing cellular responses.

These finding underline the importance of IL-17 in the human immune response against *C albicans* and, to a lesser extent, against *S aureus* in mucocutaneous areas.

In line with this, *IL-23p19* knockout mice, which are deficient in T_H17 cells, and *IL-17RA* knockout mice show an increased susceptibility to oral candidiasis, with hyphal formation and invasion of the superficial epithelial layer, partly caused by failure to recruit neutrophils to the oral mucosa, reduced levels of antimicrobial peptides, and absent fungal phagocytosis.[105]

APECED: Autoimmune regulator deficiency

Candidiasis is an eponymous clinical hallmark in a syndrome called APECED or autoimmune polyendocrinopathy syndrome type I. This rare AR disease is characterized by an early onset of the clinical triad of CMC, hypoparathyroidism, and Addison disease (adrenocortical failure). Later, endocrine autoimmune diseases develop, such as hypothyroidism, diabetes mellitus, gonadal atrophy, and hepatitis.[106,107]

APECED is caused by mutations in the autoimmune regulator *(AIRE)* gene, which has critical functions in the induction of self-tolerance.[106,107] First, AIRE allows for low-level expression and presentation of tissue-specific antigens (which are normally not expressed in the thymus) in medullary thymic epithelial cells (mTECs), resulting in deletion of self-reactive thymocytes.[108] However, some tissue-specific antigens are expressed by mTECs independently of AIRE. Yet AIRE seems to control tolerance induction even to those antigens. Hubert et al[109] showed that AIRE regulates the transfer of these antigens from mTECs to thymic DCs for indirect presentation and induction of negative selection. Furthermore, there is evidence that AIRE also influences the development of ***natural regulatory T cells*** and induction of peripheral tolerance through expression in peripheral lymphoid tissues.[108,110,111] Although AIRE-regulated central tolerance induction is most important in early infancy, AIRE-dependent peripheral tolerance mechanisms operate throughout life.[108] This might explain the occurrence of both very early-onset symptoms and autoimmunity developing during adulthood in patients with APECED.

In addition to autoreactive T cells that initiate autoimmunity, patients with APECED have been shown to have high titers of neutralizing antibodies against cytokines, including the T_H17 cytokines IL-17A, IL-17F, and/or IL-22, which is believed to be the reason for CMC in this syndrome.[89,90,112]

Puel et al[90] analyzed the plasma of 33 patients with APECED, 29 of whom had CMC, and found specific and neutralizing IgG autoantibodies against IL-17A (67%), IL-17F (94%), and IL-22 (91%) but not against other cytokines, such as IL-1β, IL-6, IL-23, and IL-26. All patients had autoantibodies against at least 1 of these cytokines. This also included patients without CMC. Kisand et al[89] evaluated 162 patients with APECED and found neutralizing autoantibodies against IL-17A (41%), IL-17F (75%), and IL-22 (91%) and severely reduced IL-17F and IL-22 responses to *C albicans* in the PBMCs of patients with CMC, which were strongly associated with neutralizing autoantibodies.

Lack of overt staphylococcal disease in most patients with APECED might result from residual IL-17 immunity, but it remains unexplained why a few patients with autoantibodies have no fungal susceptibility. However, later-identified inborn errors of IL-17 immunity (see above) strongly support the idea that functional IL-17 can protect against *C albicans* in the skin and mucosa.

SUMMARY

All signposts turn toward impaired IL-17 immunity, IL-22 immunity, or both as the cause of localized mucocutaneous fungal diseases, with defects occurring at various points along the pathway.

Fungal recognition by innate PRRs, such as dectin-1 and dectin-2, initiates a signaling cascade through Syk2 and a complex formed of CARD9/BCL10 and MALT1 that drives NF-κB responses, which leads to maturation of DCs into full effector antigen-presenting cells through upregulation of costimulatory molecules, as well as to production of proinflammatory cytokines, among them cytokines that prime naive T cells to differentiate into the T_H17 cell lineage (IL-1β, IL-6, and IL-23). These cytokines signal through the transcription factor STAT3, which in turn induces the T_H17 lineage–determining transcription factor RORγt. T_H17 cells upregulate chemokine receptors that direct migration into inflamed tissues and secrete cytokines with potent antifungal properties, such as IL-17A, IL-17F, and IL-22. These cytokines lead to production of antimicrobial peptides in the skin and mucosa and to

activation and recruitment of neutrophils through activation of epithelial cells. Neutrophils mediate microbial killing through phagocytosis, degranulation, and neutrophil extracellular traps.

Experiments with knockout mice established a basis for these insights, which have acquired validation in the human immune system through the discovery of several PIDs in patients with susceptibility to fungal infections over recent years.

Mucocutaneous candidiasis and dermatophyte infections can occur individually or alongside other symptoms in patients with various PIDs with diverse genetic defects.

Mutations in *CARD9* impair signal transduction for the induction of T_H17 cell–promoting cytokines on fungal recognition. In patients with *STAT3* mutations, these cytokines do not activate sufficient amounts of STAT3 for induction of the T_H17 cell lineage–determining transcription factor RORγt. Gain-of-function *STAT1* mutations shift the cellular response toward T_H17 cell–inhibiting cytokines and away from T_H17 cell–activating cytokines. In all 3 cases the result is a severely reduced proportion of T_H17 cells with reduced amounts of the cytokines IL-17 and IL-22, which have potent antifungal activity at mucosal sites. Finally, mutations in *IL17F* and *IL17RA* in patients with CMC, as well as neutralizing autoantibodies against IL-17 and IL-22 in patients with APECED, directly impair IL-17 and IL-22 immunity.

Clinical implications: The delineation of the critical pathways in human host defense against fungi and against *Candida* species in particular will not only lead to an improved risk stratification in affected patients (eg, by means of genetic counseling) but will also lead to improved novel therapeutic management strategies by strengthening the IL-17/IL-22 axis in patients at risk for or already having overt disease.

In patients with recurrent infections, it is very important to obtain a detailed family history and explicitly ask for the possibility of consanguinity. In patients with CMC and an AD trait, mutations in *STAT1* are the most frequent cause for the phenotype, and genetic diagnosis should be pursued and genetic counseling offered. In patients with a suspected AR trait, mutations in various genes (eg, *CARD9* and *IL17R*) might be analyzed. In patients with recurrent fungal infections, the patient's history needs to include any signs of autoimmune phenomena. The determination of T_H17 cell numbers in peripheral blood is a challenging test but might help identify patients with an underlying genetic condition. To what extent the above observations will be relevant for more common clinical problems, such as female subjects with recurrent vaginal thrush, still needs to be determined.

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