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Immunity to Commensal Fungi: Detente and Disease

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Abstract

Fungi are ubiquitous in our environment, and a healthy immune system is essential to maintain adequate protection from fungal infections. When this protection breaks down, superficial and invasive fungal infections cause diseases that range from irritating to life-threatening. Millions of people worldwide will develop invasive infections during their lives, and mortality for these infections often exceeds 50%. Nevertheless, we are normally colonized with many of the same disease-causing fungi (e.g. on the skin or in the gut). Recent research is dramatically expanding our understanding of the mechanisms by which our immune systems interact with these organisms in health and disease. In this review we will discuss what is currently known about where and how the immune system interacts with common fungi.

Keywords

Candida; *Malassezia*; *Aspergillus*; microbiome; Dectin-1; innate immunity

Introduction

Fungi are ubiquitous in the environment, being found on every continent, in the deep sea, in the air, and arctic soil. Mammals have evolved in the presence of diverse fungi, and our immune systems are adapted to their presence. We have innate pattern recognition receptors in our genomes that provide defense against fungal infection (1, 2). We make natural (generated without the requirement for exogenous antigenic stimulation) antibodies that recognize fungi (3). We have harvested fungi for food, fuel, and medicines. Curiously, studies on the microbiota associated with our bodies in health and disease have focused largely on bacteria. Recently, investigators are becoming aware that fungi are a normal part of the microbiota associated with our bodies, be it on the oily skin or the anaerobic gut (4, 5). Interactions between the immune system and these fungi (the “mycobiome”) are being recognized as important for immune homeostasis and for pathology during disease. In this review, we will focus our discussion on host interactions with the fungi that are commonly and naturally part of our microbiota, be it in the context of health or disease. For discussion

of immune responses to more overtly pathogenic fungi such as *Cryptococcus*, *Histoplasma*, *Coccidioides* or *Mucorales*, we direct the reader to other excellent reviews (6–9).

Who are commensal fungi?

The types of fungi that are reported to be associated with the body depend on the site and the methods of detection, although it is clear that representatives of the major fungal phyla, Ascomycota and Basidiomycota, are widely present (Figure 1). Early studies relied on culturing fungi from various anatomical sites, but such methods made little progress in really characterizing the net complexity of commensal fungal communities. DNA-based methods have enabled culture-independent detection and identification of fungi. Approaches such as restriction fragment length polymorphism (RFLP) analysis, oligonucleotide fingerprinting of rRNA genes (OFRG), and denaturing gradient gel electrophoresis (DGGE), were good for revealing more complexity than culturing methods, but were relatively poor at identifying specific fungi. PCR-based methods can be highly specific, but are restricted in what they can identify. High-throughput sequencing technologies can be more comprehensive, but can be challenged by efficiencies of DNA purification and amplification as well as bioinformatics and taxonomical challenges associated with identifying DNA fragments (10).

The most common targets sequenced for identifying fungi are the internal transcribed spacer regions (ITS) of ribosomal RNA genes. The first culture-independent ITS sequencing-based study to evaluate the oral mycobiome identified >80 genera of fungi, and found that *Candida* and *Cladosporium* were most abundantly detected in healthy individuals (11). Another analysis of these data identified *Malassezia* and *Epicoccum* to be more abundant (12) and highlights the situation that tools and approaches for fungal rDNA sequence analysis are still being developed and validated.

Fungi are thought to be normal commensals of the mammalian intestines. The mouse gut is home to several common fungi including *Candida*, *Saccharomyces* and *Cladosporium* species, and >50 less commonly detected genera (13, 14). Fungal populations in the mouse gut have been observed to be variable over several months, whereas the intestinal bacterial community remains relatively stable (13). In humans, sequencing approaches commonly detect *Saccharomyces* and *Candida*, with other related yeasts as well as some molds including *Penicillium* being commonly detected (15–17).

Comparing the amount of fungi to, for example, bacteria has proven challenging as it is often difficult to determine how to normalize data appropriately. 16S/ITS sequencing efforts are difficult to compare directly, especially since different types of bacteria and fungi respectively have widely different numbers of repeats of these genomic areas. Metagenomic approaches relying on assembling and identifying genomic DNA from all components of a sample may do better; in the gut, for example, shotgun sequencing efforts have suggested that fungi make up approximately 0.1% of the microorganisms (18, 19). However, fungi are much larger than bacteria, DNA recovery efficiencies are likely very different, and many fungal reference genomes are yet to be produced.

While a comprehensive catalogue of all types of fungi making up the human mycobiota would be enormous, below is a short description of some of the major types of fungi that are currently being reported:

Saccharomycetaceae

Saccharomycetaceae is a family of Ascomycetes that includes common yeasts such as *Saccharomyces* and *Candida*. *Saccharomyces cerevisiae*, baker's yeast, is found in many foods and is commonly detected in diverse sites of the body. *Candida* species including *C. albicans*, *C. tropicalis*, and *C. dubliniensis* are dimorphic, meaning that they are yeasts that can also grow in filamentous form (20). These *Candida* spp. can cause minor skin and mucosal infections in healthy individuals and can cause life-threatening mucosal and blood infections in immunocompromised people. Taxonomically, *Candida* is a complicated genus with many organisms named *Candida* not being particularly closely related to these pathogenic organisms. For example, *Candida glabrata*, a commonly-occurring disease-causing commensal yeast, is more closely related to *S. cerevisiae* than to *C. albicans*, is not dimorphic, and is more accurately classified as a *Nakaseomyces* (21, 22). Other Saccharomycetaceae commonly found in studies of commensal fungi in this group include *Pichia*, *Kazachstania*, *Debaryomyces*, *Kluyveromyces* and *Meyerozyma*.

Aspergillus

Aspergillus is a genus of common molds found world-wide that are both harmful and beneficial to people (23). For example, *A. niger* is used commercially to produce citric acid and *A. oryzae* is used to ferment starches such as in the production of Sake. In contrast, *A. flavus* is a plant and animal pathogen that produces the potent carcinogen aflatoxin, and *A. fumigatus* is a medically important opportunistic pathogen that commonly causes lung infections in immunocompromised patients. *A. fumigatus*' natural ecological niche is soil; it grows as a filamentous mass and releases ubiquitous spores into the air. Environmental studies estimate that humans commonly inhale at least several hundred *A. fumigatus* spores per day (23). These spores are small enough (2-3 μm) to reach the alveoli of the lung. *Aspergillus* are also major cause of blinding corneal ulcers, and in contrast to most other common fungal infections, occur in immunocompetent individuals (24). Like many other common fungi, nomenclature of *Aspergillus* spp. can be complicated by the existence of sexual and asexual forms of the organisms that have historically been assigned different names. There are at least eight different teleomorph (sexual form) genera associated with *Aspergillus* (anamorph, asexual form) including *Emericella* and *Eurotium* (25). Various *Aspergillus* species are commonly detected in microbiota studies of the lung and gut, but as is the case with many of the fungi discussed here, whether these are truly commensal or are simply representative of the environment is difficult to establish.

Fusarium

Fusarium is a genus of filamentous mold found ubiquitously in soil, air, and on plants. Many *Fusarium* species possess the ability to produce mycotoxins and may cause mycotoxicosis following ingestion of food that has been colonized. *Fusarium* spp. may cause local skin infections that may become invasive or disseminated in immunocompromised individuals (26). Like *Aspergillus*, *Fusarium* are also a major cause of blinding corneal ulcers in

immunocompetent individuals (24). Commonly detected commensal and infection-causing *Fusarium* include *F. solani*, *F. oxysporum*, and *F. moniliforme*.

Cladosporium

Cladosporium is another genus of filamentous, spore-forming black molds that are ubiquitous in the soil and on live or dead plant material and are among the most common fungi isolated from air (27). There are over 700 described species, many of which are plant pathogens. Unlike *Fusarium*, *Cladosporium* spp. do not produce toxins and are rarely pathogenic in humans, although they have been occasionally found associated with infections of the skin, eyes, brain, sinuses and lung (28). They are also commonly associated with allergies (29).

Malassezia

Fungi of the genus *Malassezia* are lipid-dependent and lipophilic yeast species that are part of the normal skin microbiota. There are 14 described species, and although most well-known as skin commensals, they are widely distributed, being found at deep sea vents, Antarctic soils, and plant roots (30). *Malassezia* can be involved in skin disorders including pityriasis versicolor, seborrheic dermatitis, atopic eczema, folliculitis, and dandruff. Rare invasive infections can occur, primarily in neonates or in immunocompromised patients being given intravenous lipid-rich nutrition (31). The three sequenced genomes, *M. globosa*, *M. restricta*, and *M. sympodialis*, reveal less than 5,000 genes (among the smallest of free-living fungi), and a key feature of these genomes are their incomplete fatty acids synthesis metabolic pathways. The organisms rely on secreted lipases, phospholipases, and acid sphingomyelinases to process extracellular lipids. This reliance on often diverse fatty acids for growth can make some *Malassezia* a challenge to culture (30).

Immune homeostasis with fungi

The microorganisms that occupy mucosal surfaces of the body are critical for appropriately tuning immune responses to ensure efficient responses to pathogens while limiting responses directed towards host tissues and innocuous agents such as allergens (32). Research over the last several decades has provided compelling evidence supporting a role for bacteria in maintaining immune homeostasis, however it is now becoming increasingly clear that the host immune system is also in a dynamic relationship with fungal populations that reside on mucosal surfaces, and disturbance of this relationship can also have serious immune and non-immune consequences for the host (5). Below we will detail what is currently known about the role commensal fungal communities play in modulating immune function.

Probiotic fungi

One of the first indications that fungi could play a role in modulating immune homeostasis comes from the long history of using the yeast *Saccharomyces boulardii* as a probiotic to alleviate diarrhea and prevent intestinal colonization with *Clostridium difficile* following broad-spectrum antibiotic therapy (33, 34). *S. boulardii* was originally isolated by French microbiologist Henri Boulard after a visit to Southeast Asia in the early 1900's where he observed individuals drinking tea made from skins of tropical lychee and mangosteen fruits

to alleviate symptoms of cholera. He isolated the heat-resistant yeast and named it after himself. It is now generally described as a subspecies of *S. cerevisiae* (*Saccharomyces cerevisiae* var. *boulardii*) (35).

S. boulardii is thought to mediate its effects through direct interaction with the microbiota as well as via mechanisms that engage the immune system. The yeast secretes proteases and phosphatases that inactivate toxins produced by intestinal pathogens such as *C. difficile* and *E. coli* (36, 37), and can also directly inhibit the growth and invasion of a number of intestinal pathogens including *C. albicans*, *Salmonella typhimurium*, and *Yersinia enterocolitica* (38, 39). More recent work has shown that *S. boulardii* has immunomodulatory functions that boost immunity to intestinal pathogens and limit pathological inflammation in diseases such as ulcerative colitis, Crohn's disease, and *C. difficile* colitis. *S. boulardii* boosts total intestinal IgA production as well as IgA specific for *C. difficile* toxin A, although how is still unclear (40). Supernatants from *S. boulardii* cultures can directly inhibit Toll-like receptor (TLR)-induced inflammatory responses in myeloid dendritic cells isolated from healthy and Crohn's disease patients (41). *S. boulardii* supplementation has been documented to have efficacy in maintenance of Crohn's disease remission (42, 43), as well as for symptomatic improvement in a subset of ulcerative colitis patients (44), which is consistent with the protective effects of *S. boulardii* administration in experimental mouse models of colitis (45–47). Together, this suggests that the organism likely exerts both pro- and anti-inflammatory effects (Figure 2A).

It will be important to determine if supplementation with probiotic fungi has systemic effects on the immune system as has been demonstrated with numerous members of the bacterial community. Along these lines, a recent study demonstrated that oral administration of the dietary yeast *Candida kefir* was not only protective in experimental colitis, but also protected mice from development of experimental autoimmune encephalomyelitis (EAE) (48). This was accompanied by alterations in the bacterial microflora, an increase in mesenteric lymph node regulatory T cells and reduction in lamina propria Th17 cells. *Candida kefir* (also known as *Candida pseudotropicalis*, *Kluyveromyces marxianus*) is one of those *Candida* spp. discussed above that is not particularly closely related to *C. albicans* and other pathogenic *Candida*.

Immune training by commensal fungi

Although *S. boulardii* is not a natural resident of the intestinal microflora, other strains of *S. cerevisiae* are common residents of human skin and gastrointestinal tract (49, 50). Recent work has suggested that the presence of non-pathogenic *S. cerevisiae* as a commensal (51), and potentially *C. albicans* (52), might have the ability to “train” the immune system to mount more efficient responses against pathogens. The cell wall of *S. cerevisiae*, similar to many fungi, consists of an outer layer of mannoproteins that encapsulate a network of β -1,3 and 1,6-glucans, and chitin. These polysaccharides are among targets for innate immune receptors that activate pro- and anti-inflammatory responses (Table 1). Cavalieri and co-workers observed that pre-exposure of human monocytes to particular strains of *S. cerevisiae* promoted more efficient pro-inflammatory cytokine production (IL-6 and TNF- α) and intracellular killing of bacteria and fungi upon secondary stimulation with microbes

(51). This state of “trained immunity” was due to differing amounts of chitin in the yeast strains. Pre-exposure of naïve mice to purified preparations of *S. cerevisiae* chitin induced efficient protection against a normally lethal systemic infection with *C. albicans* (Figure 2B). Interestingly, the authors also demonstrated that clinical isolates of *S. cerevisiae* from stools of healthy and Crohn’s disease patients had significantly elevated levels of chitin compared to laboratory and environmentally isolated strains.

It is intriguing to speculate that the elevated chitin content of commensal *S. cerevisiae* is important for immune system training, or that strains of *S. cerevisiae* with elevated chitin content have been selected as a part of the normal intestinal microflora for their beneficial immunomodulatory properties. Another study showed that chitin isolated from *Candida albicans* can dampen inflammation through the preferential induction of IL-10 by macrophages (53). Whether this anti-inflammatory property of chitin allows for evasion of the immune response or serves as a mechanism to promote commensalism of normal members of the fungal mycobiota remains to be determined. Furthermore, it will be important to determine if chitin recognition by the immune system plays a role in the gastrointestinal protective effects associated with *S. boulardii* supplementation.

Regulation of lymphocyte recirculation by commensal fungi

The above studies provide evidence that exogenous administration of known species of commensal fungi can modulate immune function, however there is currently limited evidence exploring the idea that indigenous populations of commensal fungi regulate the immune system under normal conditions. One important recent study reported that a unique subset of RALDH⁺ (retinol dehydrogenase enzyme; necessary for retinoic acid production) dendritic cells is recruited from the intestinal lamina propria to the mesenteric and peripheral lymph nodes (pLNs) shortly after birth, and that their arrival is dependent on the presence of commensal fungi (54) (Figure 2C). These dendritic cells were shown to be required for induction of lymphocyte adhesion molecules MAdCAM-1 and PNAd, necessary for entry of recirculating lymphocytes into lymph nodes. These dendritic cells are not recruited to lymph nodes in germ-free mice, and this causes the abnormal structure and reduced cellularity of secondary lymphoid organs that is observed when mice lack microbial colonization (55). The authors observed that treatment of specific pathogen-free (SPF) mice with antifungal drugs resulted in significant reduction in this dendritic cell population and that exogenous administration of a known mouse commensal fungi, *Candida tropicalis* (13, 14), was sufficient to increase this population in pLNs. Together the data support the conclusion that the normal presence of commensal fungi has a significant role in maintaining immune homeostasis. Whether these dendritic cells are directly interacting with commensal fungi to promote pLN recruitment is currently unclear, however it was recently shown that a population of Peyer’s patch-associated Langerin-positive dendritic cells in the small intestine are able to sample orally administered *C. albicans* and *C. tropicalis* (56) (Figure 2C). It will be interesting to determine whether RALDH⁺ dendritic cells directly contact commensal fungi to promote their subsequent recruitment to the periphery or if they indirectly facilitate this process.

In related work, Wheeler et al. observed that oral treatment of mice with antifungal drugs resulted in increased disease severity in acute and chronic models of colitis, and exacerbated the development of allergic airway disease (57). Microbiota profiling revealed that the drugs induced restructuring of fungal communities, characterized by reduced representation of *Candida* spp. and a commensurate increase in *Aspergillus*, *Wallemia*, and *Epicoccum* spp. Furthermore, oral supplementation with a mixture of three fungi found to expand during oral antifungal treatment (*Aspergillus amstelodami*, *Epicoccum nigrum*, and *Wallemia sebi*) was sufficient to recapitulate the exacerbating effects of antifungal drugs on allergic airway disease. Interestingly, these fungi did not induce the changes necessary to recapitulate the increase in disease severity in the acute model of colitis. Together these results indicate that disruption of commensal fungal populations can influence local and peripheral immune responses, although whether a “healthy” commensal fungal population is necessary to balance or modulate bacterial populations or is directly necessary to interact with immune cells (or both) is not yet clear.

Immune tolerance induction by commensal fungi

The interaction between commensal and/or environmental microorganisms and the immune system in normal healthy individuals is thought to promote a “peaceful coexistence” between host and microbe. It is therefore logical to suppose that some commensal fungi would evolve mechanisms to promote their tolerance by the host immune system in order to establish colonization. In the case of *Candida*, investigators have noted that peripheral blood from healthy human donors contains an expanded population of regulatory T cells specific for the commensal *C. albicans*, which outnumber *C. albicans*-specific T effector cells by 5-10 fold (58). Recognition of *C. albicans* by mouse intestinal Peyer’s patch dendritic cells induces a tolerogenic phenotype in the dendritic cells that is dependent on fungal induction of 2,3 indoleamine dioxygenase (IDO) (59). IDO expression by tolerogenic dendritic cells is important for metabolizing the amino acid tryptophan into substrates that activate the aryl-hydrocarbon receptor (AhR) in T cells to promote regulatory T cell generation (60, 61) (Figure 2D). Further, investigators observed that *C. albicans*-induced tolerogenic dendritic cells were protective during experimental colitis in mice (59).

Sensing of immune status by commensal fungi

While we typically think of commensal microbe-immune interactions in terms of immune cells responding to microbial stimuli, there is also evidence that commensal fungi respond to the immune status of the host and that this is an important determinant of commensalism versus pathological fungal invasion. During the course of intestinal colonization, *C. albicans* modulate expression of a transcription factor (Efg1p) that normally regulates expression of a number of cell wall components as well as genes important for yeast-hyphal transition. Upon colonization of the intestines in wild type mice, *C. albicans* mRNA levels of *EFG1* were higher than in *C. albicans* colonizing T cell-deficient nude mice, suggesting that immunological factors influence the expression of *EFG1* (62, 63). Yeast lacking EFG1 could colonize wild type mice initially, but are rapidly lost, suggesting that upregulation of EFG1 is important for adaptation to an immunocompetent environment.

While it is not yet clear how T cell-derived factor(s) influence commensal mycobiota, at least one report suggests that mammalian IL-17a binds directly to *C. albicans* and promotes fungal adhesion, aggregation, and filamentation in vitro (64). Furthermore, freshly isolated vaginal *C. albicans* stain positively for IL-17a indicating normal binding *in vivo*, and pre-exposure to IL-17a ex vivo makes *C. albicans* more virulent in mouse models of orogastric as well as oropharyngeal candidiasis. These findings provide evidence that *C. albicans*, and potentially other commensal fungi, have evolved to sense host immunity and respond in ways that help ensure their persistence at mucosal surfaces.

Commensal fungi as sources of disease

When the healthy balance between the immune system and commensal fungi breaks down, fungi can become pathogenic. While superficial fungal infections (e.g. of the skin or nails) are extremely common and tend to resolve naturally or with minimal intervention, invasive fungal diseases, especially common in immunocompromised individuals, are hard to treat and can have greater than 50% mortality even in the presence of modern care and antifungal drugs. Worldwide, *Candida* and *Aspergillus* are responsible for more than 800,000 life-threatening infections every year (65). Many fungal species found as part of the normal mammalian microbiome can be associated with diseases; however, establishing whether the pathogenic fungi are truly derived from commensal strains is often difficult to establish.

Overgrowth of *Candida* is associated with skin infections, vaginal infections, oropharyngeal infection, gastrointestinal, and blood infections. After some common bacteria, *Candida* are the fourth most frequent cause of hospital-acquired blood infections (66). In one recent prospective study of over 100 patients admitted to an intensive care unit, investigators noted a strong association between commensal *Candida* strains (identified by microsatellite profiling) and strains eventually causing invasive candidiasis (67). Similar previous studies have noted that disease causing strains match commensal strains carried by patients (68–70). Invasive candidiasis is associated with the presence of catheters and recent surgery, procedures involving breaching of the barrier between blood and *Candida*-colonized surfaces, as well as administration of broad-spectrum antibiotic therapy, which allows for overgrowth of commensal fungi (71).

Although *Candida* are a normal part of the vaginal microbiome, 5–8% of adult women will experience recurrent vulvovaginal candidiasis (72). The recurrent nature of such infections strongly suggests that the commensal strains cause disease, not consistent re-introduction of foreign strains. Further, vulvovaginitis due to *Candida* is often associated with use of antibiotics which deplete competing bacteria and presumably allows overgrowth of commensal organisms.

As noted above, *Malassezia* is a normal resident of the skin and has been implicated in diverse skin disorders (31). Requiring lipids for growth, *Malassezia* are more associated with oily areas of the skin (Figure 3A). While it is presumed that *Malassezia* strains found associated with skin disease are derived from the normal commensal strains, few studies have actually tried to establish this. Numerous studies have failed to associate specific species with disease; there is substantial geographic variability in which species are

associated with human skin, but diseases seem to be associated with increased prevalence or density of these same species (31). In a recent study of patients with seborrheic dermatitis, investigators established using PCR-based detection that *Malassezia restricta* and *Malassezia globosa* are more prevalent in patients with disease compared to controls, although they are found commonly on skin of healthy people (73). More careful sequencing-based analysis of rRNA gene regions revealed that specific strains of the yeasts associated with disease were distinct from common healthy strains, suggesting that disease might be associated with colonization by new strains. Still, whether disease is associated with *Malassezia* growth/colonization or caused by it remains unclear.

Association of *Malassezia* with disease is not restricted to the skin. Systemic disease is a consequence of catheter-associated fungal growth that typically develops in catheters used to deliver lipid-rich emulsions as in neonates receiving parenteral nutrition (74). While it is tempting to postulate that the disease-causing fungi originate in the skin, such blood stream infections seem to be largely restricted to *Malassezia furfur*, a species that is relatively rare on the skin (50, 75). Using analysis of rRNA gene regions, Kaneko et al. noted that *M. furfur* strains found on the skin around the infected catheter entrance sites matched the strains isolated from blood (74). However, whether this really means that the infection originated from the skin is not clear.

Immunity to fungal infections in the blood

Systemic candidiasis is the most common type of fungal bloodstream infection documented in the developed world and is thought to originate commonly from translocation of gut fungi into the blood (71). More than a dozen species of *Candida* have been documented to cause bloodstream infections in humans of which 95% are caused by *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* (76). The mechanism through which *Candida* disseminates from the intestines to the blood is thought to require three initiating events involving 1) intestinal colonization with *Candida* 2) defects in phagocytic cell function and/or numbers, and 3) intestinal barrier disruption (77) (Figure 2E–G). One of the most common examples where this occurs is in cancer patients receiving chemotherapy. In many cases these patients are treated with antibiotic cocktails which can induce dysbiosis of the normal intestinal microbiota, and pathological overgrowth of commensal *Candida* spp. (hit 1). The combination of *Candida* overgrowth with chemotherapy-induced neutropenia (hit 2) and mucositis (hit 3) results in a highly permissive environment through which *Candida* can disseminate to the bloodstream. Other cases are thought to arise from contamination of intravenous catheters (which may or may not come from skin resident commensals). In these situations, patients usually also present with defects in phagocytic cells, however access to the blood stream is provided directly from contaminated catheters, and likely does not require additional disruption of mucosal barriers (71).

It is well-accepted that the innate immune system is essential to control systemic *Candida* infections. There is growing evidence that the adaptive immune system plays an important role in controlling systemic infections, although patients with defects in adaptive immunity are not necessarily more susceptible to invasive disease. Below we will review what is

known about the innate and adaptive immune components necessary for limiting systemic fungal infections.

First line of defense: Serum Factors

Activation of the complement system by *Candida* spp. entering the bloodstream is a critical early factor in limiting dissemination and for priming the innate immune system for fungal phagocytosis and antimicrobial activity (78). Although humans with genetic deficiencies in components of the complement system are not more susceptible to invasive *Candida* infections (79), there is considerable evidence from mouse studies and in vitro studies with human cells that complement does play an important role antifungal immunity. *Candida* spp. are known to trigger all three pathways of complement activation which promote innate immunity against fungi through opsonization to promote phagocytosis, induction of pro-inflammatory cytokine production, and directly enhancing antimicrobial activity of recruited phagocytes. Activation of the C3 convertase gives rise to the C3b cleavage product that opsonizes and facilitates phagocytosis of fungi by neutrophils and macrophages via the complement receptor CR3 (78). Furthermore, the cleavage products C3a and C5a function as anaphalotoxins that induce pro-inflammatory cytokine production, inflammatory cell recruitment, and enhance antimicrobial activity of phagocytes (78). Importantly, mice deficient in C3 or C5 (precursors for C3a/b and C5a respectively) are highly susceptible to systemic *Candida* infections, as are mice deficient in the phagocytic receptor CR3. CR3 is unique because in addition to recognizing complement opsonized fungi, it also recognizes Pra1 and β -glucans on the surface of *C. albicans* to facilitate internalization (80, 81).

Innate immune cells that limit systemic *Candida* infections:

Neutrophils.—Neutrophils are widely considered the most important innate immune effector cell needed for early control of bloodstream *Candida* infections. Neutropenia is a major risk factor for development of invasive *Candida* infection in both mice and humans (77, 82). Furthermore, mutations in genes associated with various aspects of neutrophil recruitment and antimicrobial activity including CXCR1, myeloperoxidase (MPO), and NADPH oxidase are associated with increased risk of systemic *Candida* infections in humans (83–85). The ability of neutrophils to phagocytose fungi, undergo respiratory burst, secrete antimicrobial granules, and release neutrophil extracellular traps (NETs; decondensed DNA complexed with antimicrobial peptides released to trap and kill pathogens) make them highly toxic to fungi (86). Human neutrophils are also able to prevent yeast to hyphal transition, which is a major virulence factor during infections with *C. albicans* (87, 88).

In mouse models of disseminated candidiasis, the kidney is the major target organ affected. Neutrophils are rapidly recruited in large numbers to other peripheral organs such as the spleen and liver within 24 hours of infection, which limits fungal growth at these sites. In contrast, for reasons that are not clear, neutrophil recruitment to the kidney is significantly delayed, which can lead to uncontrolled fungal proliferation in this tissue (89). Regardless, the presence of neutrophils in the kidney is essential for controlling *Candida* infection. A recent study showed that expression of the chemokine receptor Cxcr1, while not required for neutrophil recruitment to the kidney, was essential for neutrophil degranulation and fungal

clearance at this site (85). Consistent with this observation, the authors observed that humans with a mutation in CXCR1 (*CXCR1-T276*) have impaired neutrophil degranulation and are more susceptible to disseminated candidiasis.

Despite their essential anti-fungal properties, excessive neutrophil recruitment and/or activation can also have pathological consequences. Sustained recruitment of neutrophils to the kidney, due to organ-specific production of CCR1 ligands at later times after infection, was shown to be pathogenic in mice, and this contributed to mortality in response to disseminated *Candida* infection (90). Furthermore, this study showed that CCR1-deficient mice have impaired late recruitment of neutrophils to the kidney and are actually protected from disseminated *Candida* infection due to less severe immunopathology.

Monocytes/macrophages.—Compared to neutrophils, the role of monocytes and macrophages at early times during bloodstream *Candida* infection is less clear. Monocytopenia is not a risk factor for invasive *Candida* infection in humans (91). Also, although monocytes are able to internalize and kill *Candida* spp., they are less effective than neutrophils (92). However, early work demonstrating that monocyte/macrophage depleted mice are more susceptible to disseminated candidiasis suggests an important role for these cells in anti-fungal host defense (93). The role for monocytes in killing *Candida* in the bloodstream is likely secondary to neutrophils; however, in peripheral organs such as the kidney where fungi frequently disseminate, monocytes and macrophage appear to play an important protective role. In a detailed analysis of the different leukocyte populations that accumulate in peripheral organs during invasive candidiasis in mice, Ngo et al. observed that inflammatory monocytes rapidly accumulate in the kidney, liver, spleen, and brain within 24 hours of infection (94). Furthermore, the investigators found that diphtheria toxin-mediated depletion of CCR2⁺Ly6C⁺ inflammatory monocytes in mice impairs fungal clearance in the brain and kidney, and leads to decreased survival following invasive *Candida* infection.

Tissue resident monocytes/macrophages have also recently been shown to play an important role during the early stages of systemic *C. albicans* infection. Deficiency in the chemokine receptor CX3CR1, which is highly expressed on kidney resident macrophages, resulted in impaired fungal clearance and increased mortality following systemic *C. albicans* infection (95). This was due largely to reduced macrophage survival in the kidney. This study further demonstrated that an inactivating mutation in *Cx3cr1* (*Cx3cr1-M280*) is associated with increased susceptibility to systemic candidiasis in humans.

Similar to what has been observed with neutrophils, monocytes can also contribute to immunopathology if their recruitment and/or activation is not appropriately controlled. Majer et al. observed that over-production of type 1 interferons (IFN-1) during systemic *C. albicans* infection promotes excessive monocyte activation and recruitment to the kidney. This initiates an inflammatory cascade that ultimately leads to excessive neutrophil accumulation and lethal kidney immunopathology in mice with disseminated candidiasis. Consistent with this, mice deficient in the type 1 IFN-1 receptor were also highly resistant to systemic *C. albicans* infection as a result of less severe immunopathology (96).

Dendritic cell-NK cell-neutrophil crosstalk.—Recent studies have shed light on an unexpected relationship between dendritic cells (DCs) and natural killer (NK) cells in limiting disseminated candidiasis in mice. Mice deficient in IL-17a are susceptible to systemic candidiasis, which was initially thought to be due to defective Th17-mediated adaptive immunity (97). However, further investigation revealed that the heightened susceptibility of these mice was not dependent on T cells, but was rather due to a cell-intrinsic defect in NK cell function. The investigators noted that NK cells lacking the IL-17a receptor were functionally impaired (reduced target cell lysis and IFN γ production), and also failed to produce the GM-CSF that is needed to promote neutrophil antimicrobial activity against *C. albicans* infection. In a subsequent study investigators found that mice with DC-specific deficiency in the kinase Syk, required for signaling downstream of multiple antifungal C-type lectin receptors (Dectin-1, Dectin-2, MCL, and Mincle), were highly susceptible to systemic *C. albicans* infection (98). In this case, Syk-dependent production of IL-23p19 by dendritic cells in response to *C. albicans* was necessary to stimulate NK cells to promote GM-CSF production and enhance neutrophil antimicrobial activity (Figure 2G). In contrast to the previous study, however, investigators found that neither IL-17a nor IL-17f was able to directly induce NK cell production of GM-CSF. The reason for this discrepancy is unclear; however, both studies shed light on new and underappreciated roles for DC-NK cell-neutrophil dialogue in coordinating innate control of systemic *C. albicans* infection. In humans, NK cells have been reported to have both direct (perforin-mediated) and indirect (neutrophil priming-mediated) anti-*Candida* effector functions (99). Whether a similar crosstalk with dendritic cells exists in humans remains to be determined.

Role of adaptive immunity in systemic *Candida* infection

While there is considerable evidence pointing to a protective role for the adaptive immune system during mucosal fungal infection, such as in patients with chronic mucocutaneous candidiasis (CMC, detailed below), the role of adaptive immunity in bloodstream *Candida* infections is less clear. This is highlighted by the fact that individuals with defects in T and B lymphocyte function are not more susceptible to invasive *Candida* infections (100). However, given that one of the primary risk factors for systemic *Candida* infection is fungal colonization of mucosal surfaces, defects in adaptive immunity that promote mucosal infection with *Candida* could predispose these individuals to developing disseminated disease. Notably, mice deficient in critical components of Th1 immunity such as IFN- γ and IL-18 are more susceptible to disseminated candidiasis (101–103). In addition, a recent study found that IL-6 deficient mice, which have expanded Th1 differentiation during *C. albicans* skin infection, were protected from a secondary systemic *C. albicans* infection, indicating a potentially important role for Th1 immunity in memory responses to systemic *Candida* challenge (104, 105). Humans with polymorphisms in IL-12b develop more persistent fungemia during invasive candidiasis (105). Therefore, while not necessarily an initiating risk factor, defects in adaptive immunity to *Candida* can potentially influence the progression of disease in patients with invasive candidiasis.

Immunity to fungal infections of the skin

The skin is the largest organ of the human body and plays a multifaceted role in providing barrier protection against environmental insult and microbial invasion. It is a compartmentalized organ comprised of the epidermis, dermis and hypodermis layers populated with both hematopoietic and non-hematopoietic cells. The skin harbors a microbiome composed of bacteria, fungi, viruses and parasitic mites with the highest diversity of bacteria and fungi found on drier and exposed sites (50, 106, 107). Microbial communities of the skin contribute to the orchestration of local conditions that establish a symbiotic relationship with the host (108). For example, germ free mice fail to mount a T cell-mediated adaptive response against *Leishmania major* which was reversed by colonization with the bacterial skin commensal *Staphylococcus epidermidis* (109). Below we will focus on the interaction between *Malassezia* and *Candida* spp. with the host cutaneous immune system.

Malassezia and host defense

As noted above, *Malassezia* spp. are the dominant fungal member of the skin microbiota and are dependent on processing of external lipids for growth. As such, they thrive on sebaceous gland secretions (Figure 3A). The yeast secrete enzymes necessary for fatty acid metabolism, and a consequence of this is the production of short chain fatty acids that have direct anti-microbial activity in-vitro against bacteria and fungi found on the skin (Figure 3B). One such example is azelaic acid that exhibits antimicrobial activity at lower pH conditions consistent with the pH of normal skin (110). Similarly, ethyl ester derivatives produced by the esterification of medium chain fatty acids by *Malassezia* have in-vitro anti-mycotic activity (111). In the gastrointestinal tract, short chain fatty acids produced by commensal microbes, as a by-product of carbohydrate fermentation, or by dietary supplementation promotes production of colonic T-regulatory cells (112–114). It is interesting to hypothesize that such regulatory mechanisms could also be induced as a consequence of *Malassezia* fatty acid metabolism in the skin.

The carbohydrate composition of the cell wall of *Malassezia* is different from other common fungi in that the main cell wall component is β -(1,6)-glucans instead of the Dectin-1 ligand, β -(1,3)-glucan (115). C-type lectin receptors are still involved in immune responses to *Malassezia*, but this is largely via Dectin-2 and Mincle (116). Dectin-2 recognizes hydrophilic O-linked mannanose-rich glycoproteins, while Mincle recognizes hydrophobic glucosyl- and mannosyl-glycolipids (116). Similarly, *Malassezia* has been observed to activate Toll-like receptor 2 (TLR2) in mast cells (117). Still, the relative roles that these (and other pattern recognition receptors) play in *Malassezia*-induced commensalism and disease processes are not yet well understood.

Malassezia species have been reported to produce potent aryl hydrocarbon receptor (Ahr) ligands namely indirubin, malassezin, formylindolo[3,2-b]carbazole, indolo[3,2-b]carbazole, pityriazepin, pityriacitrin and tryptanthrin (118–121) (Figure 3B). The aryl hydrocarbon receptor (Ahr) is an orphan nuclear receptor that belongs to the family of basic helix-loop-helix transcription factors and has been shown to positively influence wound healing and contribute to overall skin homeostasis (122–124). It is expressed by diverse cell

types in the epidermis leading to the hypothesis that *Malassezia*-induced Ahr stimulation may promote epithelial cell health and protection from ultraviolet radiation (31). Among CD4⁺ T cells, Ahr expression in Th17 cells has been shown to increase the production of the proinflammatory cytokines IL-17 and IL-22 in this T cell subset (125). Also, TLR-induced responses in phagocytes are inhibited by Ahr ligands (126), and Ahr plays a crucial role in the homeostasis of cutaneous invariant $\gamma\delta$ T cells (127). Thus it is also interesting to hypothesize that *Malassezia*-induced Ahr stimulation in the skin may directly modulate immune activation in health and disease (Figure 3B). In patients with atopic dermatitis, the diversity of *Malassezia* species is less than in healthy controls (128). The same observation holds true for non-*Malassezia* yeasts and filamentous fungi (128), which suggests that a decrease in diversity may alter the niche in a way that is conducive to disease.

Cutaneous *Candida albicans* infections

As previously mentioned, *Candida albicans* is a dimorphic and opportunistic fungus capable of colonizing healthy human skin. In the stratum corneum, the upper most cornified and avascular layer of the epidermis, *Candida albicans* exists mainly in its yeast phase (Figure 3C). In the stratum corneum *Candida* is exposed to antimicrobial peptides such as β -defensins and LL-37 that have anti-fungal activity (129). At least one fungal defense against these peptides is the observation that secreted aspartic proteases (SAPS) released by *Candida* can cleave and inactivate LL-37 (130). *Candida albicans* binds keratinocytes (HaCaT cell line) at least in part through interaction between Gpm1 (a fungal Factor H/FHL1-binding protein) and host vitronectin, and binding is associated with induction of proinflammatory cytokine production (131, 132). However, while keratinocytes express PRRs, in vivo evidence of their significance remains to be reported. Recently work by Kaplan et al. has uncovered a role for keratinocytes in licensing epidermal residency to Langerhans cells and tissue resident memory CD8⁺ T cells through the activation of latent TGF β (133).

Transition of *Candida* from the yeast into a pseudo-hyphal form confers the capacity to invade deeper sites such as the dermis and internal organs where they encounter diverse immune cells. Langerhans cells (LCs) reside in the epidermal region while dermal dendritic cells (dDCs), expressing either CD103 or CD11b among other cell surface markers, reside in the dermal layer (134). LCs are capable of inducing CD4⁺ Th17 cell differentiation through the production of IL-23, IL-6 and IL-1 β (135). LCs drive CD4⁺ Th17 T cell differentiation in response to budding yeasts by a mechanism involving ligation of Dectin-1 and production of IL-6 but not IL-1 β and TGF β (104). Protection for secondary fungal challenge in the skin requires Th17 but not Th1 immunity (104).

Penetrating *Candida* hyphae are morphologically altered to conceal cell wall β -glucan that could be recognized by Dectin-1, and they encounter dDCs in the dermal layer that consequently induce Th1 and CTL differentiation by producing higher levels of IL-12 and IL-27 without any IL-23 production and only very low levels of IL-1 β and IL-6 (135). Protection from secondary systemic challenge with *Candida* requires Th1 but not Th17 immunity (104). CD11b⁺ dDCs in the skin are found in close proximity to nerve fibers, and nociceptive neurons expressing transient potential cation channel subfamily V member 1 (TRPV1) are able to sense *Candida albicans* and stimulate release of CGRP, a neuropeptide

with a role in the transmission of pain (136). In this case, CGRP appears to target CD11b⁺ dDCs and induce production of IL-23, promoting expansion of IL-17 producing $\gamma\delta$ T cells which help recruit neutrophils and induce antimicrobial peptide production by keratinocytes (Figure 3C). How this sensing by nociceptors occurs is not clear, although von Andrian and coworkers recently showed the same role for these nerves in an imiquimod-stimulated model of psoriasis-like inflammation in the skin, suggesting that the fibers respond either directly to TLR agonists or to inflammatory mediators produced by other cells in response to TLR agonists (137).

Immunity to fungal infections of the gastrointestinal tract

Infections of the oral cavity

The oral cavity, much like the architecture of the skin, is composed of stratified squamous epithelium with a tough keratinized layer lining the masticatory mucosa and non-keratinized squamous epithelium lining the rest of the oral cavity. Under a normal physiological state, the oral cavity harbors a diverse mycobiota with *Candida* species found in up to 75% of healthy people surveyed (11). Development of oral *Candida* infections, such as oral pharyngeal candidiasis (OPC), is often manifested in HIV⁺ individuals with marked reductions in CD4⁺ Th17 cell numbers (138). This suggests that normal interactions between commensal fungi and the host immune system are essential to maintain the balance between commensalism and disease. Further, Ghannoum et al. noted an inverse relationship between *Candida* colonization and abundance of *Pichia* yeasts in the oral cavity of HIV⁺ patients, suggesting that fungal competition might also be promoted in different immune environments (139). *Pichia* was able to inhibit *Candida* by limiting availability of nutrients, fungal growth and expression of virulence factors (139).

Naglik and coworkers have recently identified a novel virulence factor that aids *Candida albicans* invasion during oral infection (140). They reported that *Candida albicans* strains lacking the cell elongation 1 gene (ECE1), a secreted protein expressed during filamentation, form hyphae normally, but fail to penetrate epithelial layers. They determined that this protein is proteolytically processed to generate a pore-forming toxin that is able to kill epithelial cells and that this activity is necessary for tissue penetration. This is the first report of a pore-forming yeast toxin which the investigators named candidalysin (140). It will be interesting in coming years to see what, if any, role this toxin plays in other kinds of infection or whether it participates in competition with other microbes as well.

Chronic mucocutaneous candidiasis

Chronic mucocutaneous candidiasis (CMC) is a variegated group of conditions characterized by persistent or recalcitrant superficial infections caused mainly by *Candida albicans*. Development of systemic dissemination is uncommon. These infections typically occur in the oral and genital mucosae, skin and nails (141), and the underlying mechanisms that drive these disorders are generally linked with impaired Th17 immunity (142). In humans CMC can arise from mutations in IL-17RA, IL-17RC and IL-17F and downstream IL-17R signaling adaptor Act 1 (142, 143). Patients that have mutations in *STAT3* afflicted with autosomal dominant hyper IgE syndrome (AD-HIES) have defects in T cell IL-17 and IL-22

secretion and are affected with CMC (144). Additionally, CMC has also been observed in patients with mutations to Dectin-1 and CARD9, both important for innate anti-fungal immune defense as discussed above (145, 146).

Fungi and inflammatory bowel disease

Inflammatory bowel disease (IBD) is characterized by chronic intestinal inflammation that develops due to a dysregulated immune response to commensal microbes in genetically-susceptible individuals (147). IBD is a group of diseases primarily comprising ulcerative colitis (UC) and Crohn's disease (CD). Although the exact role that the fungal microbiota plays in IBD pathogenesis is not clear, Iliev et al. have reported that a polymorphic variant of the Dectin-1 gene is associated with disease severity in patients with ulcerative colitis and that mice lacking Dectin-1 are highly susceptible to models of colitis (14). Further, antibodies to yeast (called anti-*Saccharomyces cerevisiae* antibodies, ASCA) are directed against yeast cell wall mannan and have been identified as a biomarker capable of identifying the majority of patients with Crohn's disease (CD) (148). Also, genome-wide association studies have revealed potential links between IBD and host anti-fungal immunity including polymorphisms in CARD9 and IL-23R that are associated with susceptibility to disease (149, 150). Together, the data suggest that altered immune interactions with fungi may play a significant role in at least some patients with IBD. However, whether specific types of fungi are involved and how altered immune interactions may contribute to disease initiation or persistence will require further investigation.

Several studies have begun to explore whether IBD is associated with changes in the types of fungi found in fecal samples. An early culture-based study reported enrichment of *Candida* species (specifically *C. dubliniensis* and *C. parapsilosis*) in IBD patients (151). More recent high throughput DNA sequencing-based approaches have noted reduced fungal diversity and prevalence of specific *Candida* spp. (including *C. parapsilosis*) in pediatric IBD patients (17), and *Candida albicans* in adult IBD patients (15). In the latter study, investigators noted an inverse correlation between *C. albicans* and *S. cerevisiae* which could be related to a potential homeostatic role of *S. cerevisiae* as discussed above. These investigators also noted that the presence of *Malassezia sympodialis* in stool samples was associated with polymorphisms in Dectin-1 and that an IBD-linked CARD9 polymorphism was associated with increases in *S. cerevisiae* (15). However, how or whether these changes directly affect disease and how or whether these stool-associated fungi interact with immune cells will require deeper investigation.

Future Directions

In this review, we have reflected on recent work investigating interactions between fungi and the immune system in defining healthy and disease states. In the context of health, we are only just beginning to recognize immune mechanisms involved in continuous interaction with environmental and commensal fungal communities. These interactions contribute to maintenance of a healthy immune system that is ideally balanced to allow colonization by commensal organisms but poised to induce effective host defense when challenged with infection. Future studies will provide insights into whether commensal fungal populations

and immune responses to them can be manipulated to promote health or ameliorate disease. In the context of disease, effective host immune responses are essential to protect against serious life-threatening fungal infections. Given the prevalence of serious fungal infections, and the associated mortalities, much more effort needs to be applied to understanding how the immune system optimally copes with infectious challenge and how rational strategies may be devised to strengthen these defenses.

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Figure 1. Distribution of common commensal fungi.

Fungal representation at various sites and sources are indicated. Relative prevalence across multiple studies at each site are represented by the size of the fungal genera name.

Figure 2. Interaction of commensal fungi with the mucosal immune system during homeostasis and disseminated fungal infection.

Top panel depicts normal homeostatic conditions. (A) Probiotic *Saccharomyces* boosts intestinal IgA, directly inhibits intestinal pathogen infection, and also limits pathological inflammatory responses. (B) Commensal *Saccharomyces* induces monocyte training that limits pathological *Candida* infection. (C) Commensal *Candida* being sampled by Langerin⁺ intestinal dendritic cells and RALDH⁺ dendritic cells traffic to the peripheral lymph nodes (pLN) in response to commensal *Candida* to induce lymphocyte homing molecules on pLN high endothelial venules (HEV). (D) Recognition of *Candida* by intestinal dendritic cells

induces a tolerogenic phenotype characterized by IDO expression and induction of *Candida*-specific regulatory T cells. Bottom panel depicts situation where antibiotic treatment-induced dysbiosis combined with immunosuppression leads to invasive candidiasis. (E) Antibiotic-mediated disruption of commensal bacteria promotes intestinal overgrowth of commensal *Candida*. (F) Mucosal disruption (for example following chemotherapy-induced mucositis) allows for bloodstream invasion by intestinal commensal *Candida* and dissemination to peripheral organs. *Candida* activates complement in the bloodstream which promotes neutrophil and monocyte phagocytosis and activation. (G) DC-derived IL-23p19 activates NK cells to produce GM-CSF needed for neutrophil antimicrobial activity. Neutropenia as well as polymorphisms which impair PMN and monocyte function promote full development of invasive candidiasis.

Figure 3. Interactions between fungi and the immune system of the skin.

(A) *Malassezia* species are associated with areas of the skin where sebaceous secretions are present. These fungi utilize long chain fatty acids such as sebum as required carbon sources. *Malassezia* yeasts have been associated with dermatologic conditions such as *Malassezia* folliculitis. Normally confined to the infundibulum region of the skin, *Malassezia* yeasts can invade into the hair follicle causing damage and inflammation. (B) Metabolic byproducts of *Malassezia* yeasts are important in skin homeostasis. Short chain fatty acids produced from the metabolic breakdown of sebum have dual anti-bacterial and anti-mycotic properties. Potent AhR indolic ligands produced by *Malassezia* can inhibit phagocyte responses to TLR stimulation and contribute to cutaneous invariant $\gamma\delta$ T cell homeostasis in the skin. (C)

Immune responses to *Candida albicans* skin infection are compartmentalized. In the epidermis, Dectin-1 engagement on LC by *C. albicans* yeasts stimulates IL-6 production leading to the induction of Th17 CD4⁺ T cell differentiation and skin immunity. CD103⁺ dermal DCs detect hyphae *C. albicans* and produce IL-12 thus priming Th1 CD4⁺ T cell differentiation and providing systemic protection. Skin TRPV1⁺ nociceptive nerves are able to detect *C. albicans* and respond by secreting CGRP which acts on nearby CD301b⁺ dermal DCs to produce IL-23. $\gamma\delta$ T cells respond to IL-23 stimulation by producing IL-17 which activates neutrophils and stimulates anti-microbial peptide production by keratinocytes.

Table 1.

Innate immune receptors recognizing fungi

Receptor	Predominant immune cell expression ^a	Fungal Ligands	Genetic Evidence ^b	References
Dectin-1	DCs (diverse populations), Langerhans cells, macrophages, monocytes, neutrophils, mast cells	β-glucan	<i>C. albicans</i> <i>A. fumigatus</i> <i>P. carinii</i>	(152–155)
Dectin-2	DCs (lung), Langerhans cells, macrophages, monocytes, neutrophils, γδ T cells	α-mannan	<i>C. albicans</i> <i>C. glabrata</i> <i>C. neoformans</i>	(152, 156–158)
Dectin-3 (MCL)	Langerhans cells, macrophages, monocytes, neutrophils	α-mannan	<i>C. albicans</i>	(152, 158)
Mincle	DCs (lung and adipose tissue), Langerhans cells, macrophages, monocytes, neutrophils, eosinophils, basophils	glyceroglycolipid & mannosyl fatty acids	<i>C. albicans</i> <i>M. pachydermatis</i> <i>F. pedrosoi</i>	(116, 152, 159, 160)
DC-SIGN	DCs (diverse populations), Langerhans cells, plasmacytoid DCs, macrophages, monocytes	mannan	<i>A. fumigatus</i>	(152, 161, 162)
Langerin	DCs (lung and thymus), Langerhans cells, liver macrophages	mannose, fucose, N-acetyl-glucosamine, β-glucan, mannan	N/A	(162)
Mannose receptor	DCs (lung, adipose, liver), macrophages, monocytes, γδ T cells	N-mannan	<i>C. neoformans</i>	(152, 163)
TLR1/2, 2/6	Diverse cell types including myeloid immune cells and T and B lymphocytes	phospho-lipomannan (PLM)/glucuronoxylomannan (GXM)	<i>C. albicans</i> <i>A. fumigatus</i>	(152, 164–167)
TLR4	Diverse cell types including myeloid immune cells and B cells	O-mannan	<i>C. albicans</i> <i>A. fumigatus</i>	(152, 164, 167, 168)
Galectin-3	DCs (diverse populations), Langerhans cells, macrophages, monocytes, neutrophils, activated T cells, γδ T cells, basophils, mast cells, eosinophils	β-mannosides	<i>C. albicans</i>	(152, 169, 170)
CD36	DCs (diverse populations), macrophages, monocytes	β-glucan	<i>C. neoformans</i>	(152, 171)
Complement receptor 3 (CR3)	DCs (diverse populations), Langerhans cells, macrophages, monocytes, neutrophils, eosinophils, basophils, NK cells, activated T cells, peritoneal B cells	β-glucan	<i>C. albicans</i>	(80, 152, 172)
Mannose-binding lectin (MBL)	Hepatocytes (serum factor)	mannan	<i>C. albicans</i> <i>A. fumigatus</i>	(152, 173, 174)
Pentraxin-3	DCs, macrophages, neutrophils (secreted factor)	galactomannan	<i>A. fumigatus</i>	(175–177)

^a mRNA expression (mouse) according to ImmGen database (normalized expression cutoff = 200)

^b Fungal infections (in vivo evidence) associated with genetic deficiency/mutation in mice or humans

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