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The mycobiota: interactions between commensal fungi and the host immune system

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Abstract

The body is host to a wide variety of microbial communities from which the immune system needs to protect us and which are important for normal immune system development and maintenance of healthy tissues and physiological processes. Investigators have largely focused on the bacterial members of these communities, but an increasing number of studies underscore the presence of fungi as well that may be important for defining the communities and their interactions with immune cells. In this Review we discuss what is currently known about the makeup of fungal communities on the body and features of the immune system that are particularly important for interacting with fungi at these sites.

Largely due to advances in high-throughput sequencing technologies, the past decade or two has seen enormous progress in our understanding of the prevalence and diversity of the microbial communities associated with nearly all of our mucosal surfaces. This microbiota influences processes ranging from digestion to behaviour and is increasingly being appreciated as a fundamental and necessary component of our physiology[1-4]. The innate and adaptive arms of the immune system are charged with maintaining a healthy relationship with this microbiota and are, in fact, profoundly shaped by the presence of commensal microorganisms. Microbiota has largely been used to refer to the commensal and pathogenic bacteria that inhabit our body surfaces, but recent studies have begun to indicate that other organisms, specifically fungi, are also a significant, although substantially smaller, component of this microbiota. The term mycobiota has been used to refer to this fungal component.

Commensal fungi probably have important roles in health and disease. Fungal infections are increasing in prevalence owing to more people living with suppressed immune systems due to, for example, AIDS, organ transplantation or chemotherapy, and commensal fungal populations can be the source of the opportunistic pathogens that affect these patients. Further, emerging studies suggest that commensal fungal changes may be relevant in diseases that are not primarily fungal such as cystic fibrosis or inflammatory bowel disease. Just as commensal bacteria are important for the development and tuning of the immune system and individual types of bacteria compete with each other for resources in the microenvironment, it is also likely that commensal exposure to fungi influences the immune

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system and that fungi compete with each other and bacteria for resources. Just as bacterial "**dysbiosis**" may be an important feature of disease, so too might alterations in the mycobiota.

To understand the impact of interactions between the mycobiota and the host immune system we need to know the makeup of the mycobiota in different locations of the body, and we need to understand the mechanisms by which the immune system interacts with fungi at mucosal surfaces. This Review focuses on efforts to identify the fungal microbiota at different sites, changes in fungal populations that occur together with pathologies, and how the immune system is thought to interact with specific fungi at various sites.

Fungi: an underappreciated part of the microbiota

In the context of the entire microbiota, fungi are generally considered to be a minor component. In the gut, for example, recent **shotgun sequencing** efforts have suggested that fungi make up approximately 0.1% of the microorganisms[5,6]. There are several reasons to question whether this underestimates the number and significance of fungi. First, the estimates are based on identifying sequences based on available annotated reference sequences, and fungi are highly underrepresented in these databases compared with bacteria. For example, a recent check (Feb, 2014) of the NCBI Genome database revealed 57 complete fungal genomes compared with >2,700 complete bacterial genomes. This suggests that fungi might be under-detected compared with bacteria in shotgun sequencing efforts. Second, a typical fungal cell (which is ≈5 μm in diameter) is >100 times larger than a typical bacterial cell (which is ≈1 μm in diameter). Fungi are thus a much more substantial mass of biomaterials than simple genome-counting numbers might suggest. Third, fungi, as eukaryotes, are likely to contribute unique metabolic features to the microbiota. Finally, recent studies have clearly demonstrated that even "minor" components of the microbiota that proliferate in response to diet or during dysbiosis can have profound effects on the immune system[7-9].

Approaches to studying the mycobiota

To study the mycobiota and its interactions with the immune system, the fungal part of the microbiota needs to be identified and quantified. 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. The advent of new, affordable high-throughput sequencing technologies has made direct sequencing of fungal DNA the preferred method for characterizing mycobiota. The most sensitive and affordable approach is to amplify a small variable region of fungal genomes in a sample by the polymerase chain reaction (PCR), sequence many thousands or millions of these fragments, and identify each fragment by comparison to a database. Thus which fragment is targeted is an important factor in the efficacy of the approach. Common

targets have been the 18S small subunit ribosomal DNA (rDNA), 28S large subunit rDNA or internal transcribed spacer regions (BOX 1).

The quality of data obtained through sequencing rDNA fragments depends on having a comprehensive, well-annotated database of fungal sequences to compare the fragments to. While several excellent databases have been developed for identifying bacterial 16S rDNA sequences, this is still a formidable challenge for investigators working with fungal sequences. The sequences available in GenBank probably only represent a fraction of the fungi that will be discovered; some estimates suggest that as little as 1% of fungal species are represented[10]. Furthermore, a large fraction of the available sequences are annotated as "uncultured", which is not very useful for taxonomical purposes, and sequence annotations are plagued with misclassifications. 10-20% of sequences have been estimated to be misattributed[10], and fungal taxonomy is in a state of enormous flux as sequencing data become available and more precise relationships between different fungi are characterized. Finally, it is very common for sexual and asexual forms of a fungus to be classified as different taxa (BOX 2). These are formidable barriers to investigators wanting to describe commensal fungal communities, but many approaches are being developed to address these problems, and the development of better tools will lead to improvement in the detection of fungal species.

The intestinal mycobiota

Fungi are normal inhabitants of the mammalian gastrointestinal tract[11-16]. *Candida* species have been successfully cultured from the intestines of healthy individuals, and increased *Candida* colonization of the intestine has been seen in inflammatory bowel disease (IBD) patients[17,18]. Early culture-independent studies of the mouse mycobiota using OFRG revealed a highly diverse community of fungi in mouse intestines[12]. In humans, DGGE analysis of 18S fungal rDNA revealed differential fungal profiles between healthy and ulcerative colitis patients[19]. Whereas initial studies suggested that human and mouse intestines are populated with diverse fungal microbiota, the approaches were labour intensive, marginally quantitative and could not be used to evaluate in-depth fungal variety. More recently, high throughput sequencing approaches have been used to explore fungal communities populating both human and mouse gut. These studies have shown that the gut is home to >50 genera of fungi with *Candida, Saccharomyces* and *Cladosporium* species being particularly common (Figure 1)[13,16]. With respect to *Candida* species, one study reported that *Candida tropicalis* is common in mice[13], whereas *C. albicans, C. glabrata, C. dubliniensis* and *C. parapsilosis* are most prevalent in humans[19-21]. Only a few of the most common gut fungi were found in mouse food, suggesting that the majority are indigenous to the intestine[13,14].

Studies in newborn pups and human infants have suggested that bacterial communities in the gut are initially unstable but become more stable in early childhood and develop more modestly through adulthood[22-24]. However, this may not be the case with the gut mycobiota. One recent study demonstrated that fungal populations in the mouse gut displayed episodic variation over several months, although the bacterial community remained relatively stable[14]. This suggests that fungal populations are more variable and may be influenced by

fungi in the environment. Another explanation might be that bacteria are more highly abundant than fungi and that a consequence of this is that their communities are more robust than fungal communities. Alterations in diet can also affect the fungal microbiota; in humans, plant-based diet consumption has been linked to an increase of *Candida*, whereas the consumption of an animal-based diet facilitated the expansion of *Penicillium* species[15].

Influence on bacterial microbiota

Fungal and bacterial communities undoubtedly influence each other. We have observed that commensal fungi in the mouse gut can be found in patches together with gut bacteria[13]. Germfree mice are highly susceptible to *Candida* infection[25], and antibiotic treatment supports *Candida* colonization and overgrowth in the mouse gut[14,26,27]. In one study, long-term antibiotic treatment (76 days) led to robust expansion of fungi, reaching 99% of all intestinal microorganisms detected by deep sequencing analysis[14]. This was mostly due to an expansion of *Candida* species which was the single genus found in the faeces by the end of the treatment. Similarly, prolonged antibiotic treatment in humans can predispose to fungal infections, due mostly to expansion of *Candida* species[28,29]. In keeping with the idea that fungi can also influence the bacterial composition of the gut microbiota, a recent study has demonstrated that restoration of the bacterial microbiota after treatment with a single antibiotic (cefoperazone) was strongly influenced by colonization with *C. albicans*[30]. There were significant decreases in *Bacteroidetes* and *Synergistetes* while *Firmicutes* remained unchanged. However, whether *Candida*-induced microbiota perturbation is transient or how it affects the host remains to be elucidated.

Interactions with the immune system

We know little about if or how most intestinal commensal fungi interact with the host immune system, although many innate immune receptors have been shown to interact with fungal pathogens (Figure 2). There is ample evidence that host control of intestinal fungi is important, and a variety of specific immune mechanisms have been implicated in the clearance of fungi at mucosal surfaces[31,32]. Among these, C-type lectin domain family 7 member A (Dectin-1), caspase recruitment domain-containing protein 9 (CARD9), interleukin-17 (IL-17) and IL-22 have emerged as molecules that are responsible for host defence against fungi, and genetic mutations in each of these molecules is associated with susceptibility to fungal infections in humans (Table 1). We have reported that a genetic polymorphism in *CLEC7A*, the gene encoding the antifungal receptor Dectin-1, is associated with severity of ulcerative colitis severity in humans, and have observed that experimental colitis in mice is more severe in the absence of Dectin-1[13]. The more severe colitis in mice was accompanied by fungal invasion of the colonic mucosa, as well as a general expansion of opportunistic fungi such as *Candida* and *Trichosporon* species, and a decrease in non-pathogenic *Saccharomyces* species. In patients with IBD, the disease has been reported to be associated with an enrichment for *Candida* species (specifically *C. dubliniensis* and *C. parapsilosis*), suggesting that fungal dysbiosis may accompany disease[19]. However, the DGGE method used was limited in its ability to fully characterize the fungal populations, and further studies applying more sensitive deep sequencing analysis are needed.

A deficiency in CARD9, a signalling molecule downstream of Dectin-1 and other lectins involved in fungal recognition, has been also linked to susceptibility to IBD in humans[33-35]. Although CARD9 might be involved in signalling by other receptors, its specific importance in antifungal immunity is supported by the observation that individuals with rare null mutations in CARD9 are specifically susceptible to fungal infections and no other type of infection[36]. CARD9 deficiency in mice enhances susceptibility to experimental colitis, augmented fungal burden in the gut, increased antifungal antibodies in serum and wasting disease. In addition, these mice can be partially rescued by antifungal treatment, further supporting a role for immunity to intestinal fungi being important in colitis[37].

IL-22, a cytokine that similar to IL-17 (discussed in more detail below) is closely associated with mucosal immunity, has been directly implicated in controlling gastrointestinal fungi; mice lacking IL-22 are more susceptible to gastrointestinal candidiasis when infected intragastrically with *C. albicans*[38]. Further, effective host defence required IL-12 and interferon-γ (IFN-γ, implicating an important contribution of **T helper 1 (T_H1) cell-mediated immunity**. However, whether similar mechanisms are involved during an ongoing commensal relationship with *C. albicans*, a lifetime colonizer in the human gut, remains to be elucidated. Curiously, mouse models of colitis show that both IL-17A and IL-17F are dispensable for protection against gastrointestinal *Candida* species[38].

Striking evidence suggests that intestinal *Candida* species can influence immunity at distant body sites through both interactions with immune cells and the production of fungal metabolites. Antibiotic-induced overgrowth of *Candida* species in the intestines has been shown to promote lung inflammation in a mouse model of allergy[26,39]. *Candida* species (as well as many other fungi) directly produce prostaglandin E2 (PGE2), a potent immunomodulator produced by immune cells, from host arachidonic acid, and it has been proposed that *Candida*-produced PGE2 might be involved in allergic inflammation.[40-42]. Indeed it has been recently demonstrated that *Candida*-derived PGE2 can reach the lungs through the bloodstream, act on lung macrophages and promote allergic inflammation[43]. Similarly, an association between graft-versus-host disease and gut colonization with *Candida* species has been seen in transplant patients[44]. Gut colonization was correlated with presence of a defective Dectin-1 gene, suggesting that immune-mediated regulation of colonization may have an effect on peripheral tolerance.

Oral mycobiota

The oral cavity is a well-known environment for microbial growth, although the fungal members of this community have rarely been assessed. The most complete culture-independent evaluation of the mycobiota in the healthy mouth show that the oral cavity is home >75 different fungal genera, with *Candida, Cladosporium, Aureobasidium, Aspergillus*, and *Fusarium* being among the most common[45]. The nature of the host immune response to normal fungal carriage in the mouth has not been evaluated. However, it is well-known that immunosuppressed individuals (such as patients with HIV or undergoing chemotherapy) frequently develop oropharyngeal candidiasis, demonstrating that local immunity is necessary to contain at least *Candida* species. Whether local immunity influences colonization or growth of other fungal species is not known.

Genetic studies in humans provide some hints as to the types of immunity that are necessary for healthy containment of *Candida* species in the mouth[46]. Chronic mucocutaneous candidiasis is a condition characterized by recurrent *Candida* infections of the mouth, skin, and other mucosal surfaces. Several groups have recently demonstrated that mutations in signal transducer and activator of transcription 1 (encoded by *STAT1*), a signalling molecule important for responses to IFNγ, IL-17, and IL-22, render patients highly susceptible to chronic mucocutaneous candidiasis with all cases exhibiting oral pathologies[47-50]. In addition, mutations in the genes encoding IL-17RA, IL-17F, and adapter protein CIKS (ACT1), a protein necessary for IL-17R signalling, have been associated with chronic mucocutaneous candidiasis (Table 1)[51,52]. This susceptibility to oral *Candida* infection can also be caused by an autoimmune disorder called autosomal recessive autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) in which mutations in *AIRE* lead to the production of autoantibodies against IL-17A, IL-17F, and IL-22[53]. In mice, deficiencies in the IL-17 pathway have also been shown to be more susceptible to oral candidiasis[54,55]. Together, these findings highlight the remarkably selective importance of Th17 immunity in restricting pathology caused by *Candida* colonization.

The innate responses that drive this Th17 immunity can also be inferred from genetic studies. People with genetic deficiencies in CARD9 are highly susceptible to invasive *Candida* infections, typically accompanied by a history of oral infections[36,56,57]. Notably patients with CARD9 deficiency showed decreased numbers of Th17 cells[36]. In a mouse model of oral *Candida* infection it was recently confirmed that CARD9 is necessary for mounting a Th17 cell response, although IL-17 dependent innate responses do not involve CARD9[58]. Of the receptors that might activate CARD9, the role of Dectin-1 has been explored in a mouse model and was found to be important for effective defence[59]. A common premature stop mutation in Dectin-1 in humans has been associated with **onychomycosis**, not oropharyngeal candidiasis[60]. This might suggest that Dectin-1 is not involved in oral antifungal immunity in humans, although the prevalence of the null mutation is sufficiently high that the penetrance of the effect must be low, even for onychomycosis, and nothing like the severity of the CARD9 mutations. Additional fungal-sensing receptors such as Dectin-2 can signal through CARD9, and this redundancy might explain the more severe oral disease in the absence of CARD9. Further studies will be required to determine the role of additional receptors such as Dectin-2 in oral antifungal immunity.

Skin mycobiota

The skin surface is the first point of contact of the body with bacteria and fungi coming from the environment and is home to billons of microorganisms that have developed a commensal relationship with the host. Deep sequencing approaches have demonstrated that skin harbours its unique bacterial and fungal microbiota[61-65]. Further, the skin microbiota depends on the nature of the skin site, with certain patterns associated with moist, dry or sebaceous microenvironments[61,62]. As the skin is a self-renewing organ, dead cells are continuously shed, providing an environment for saprophytic microbial growth.

Culture-dependent approaches have identified many commensal fungi associated with skin, with *Malassezia* as the most common genus, followed by *Penicillium* and *Aspergillus*[63,66]. Other fungi such as *Alternaria, Candida, Rhodotorula, Cladosporium* and *Mucor* have also been cultured, but with a lower frequency[63,66]. Culture-independent sequencing studies confirmed that the genus *Malassezia* most commonly represented across different body sites of healthy human skin[63,67,68]. The higher resolution of these approaches allowed identification of specific species, and certain *Malassezia* species are associated with specific body sites[63]. *Candida* species were also detected across the body sites and were mostly represented by *C. tropicalis, C. parapsilosis* and *C. orthopsilosis*, species different from those typically populating the human gut. While sites on the foot revealed the greatest fungal diversity (40 to 80 genera), other sites such as nare, glabella, back, manubrium, and palm had greater bacterial than fungal diversity[63]. This reverse correlation between bacterial and fungal community diversity might be due to the presence of specific nutrients at different body sites and point to a complex interaction between the two communities.

Patients with primary immunodeficiencies suffer from recurrent fungal infections. Although the immunopathology of each specific immunodeficiency differs, a common hallmark of many is the development of atopic dermatitis-like eczema. One recent study in patients with primary immunodeficiencies revealed a direct link between host immunity and its effect on the skin bacterial and fungal microbiota[69]. Pathways including STAT3 and dedicator of cytokinesis protein 8 (Dock8) (leading to hyperimmunoglobulin E syndrome) and WAS/WASL-interacting protein family member 1 (WAS, leading to Wiskott-Aldrich syndrome) were affected, and patients exhibited altered bacterial community structure, increased fungal diversity and increased abundance of *Candida* and *Aspergillus* species in the skin (Table 1)[69]. Similar results were obtained in another study showing that non-*Malassezia* fungal microbiota diversity is increased in patients with atopic dermatitis, with augmented representation of *Candida, Cryptococcus* and *Cladosporium* species[68]. Analysis of co-occurrence of specific bacteria and fungi suggested that the two communities might interact[69].

An autosomal recessive *CARD9* mutation can lead to deep dermatophytosis, which in contrast to a superficial mucocutaneous disease, affects dermal and subcutaneous tissue, lymph nodes and, occasionally, the central nervous system leading to mortalities[56]. As noted above, these patients have decreased numbers of Th17 cells[36]. In vivo studies have shown that mice deficient in IL-17A or IL-23 are more susceptible to *C. albicans* skin infection[70]. Although IL-22 deficiency seems to play a role in mucocutaneous dieses in humans, it appears to not be involved in the mouse model of *Candida* skin infection[70]. Future studies will be needed to elucidate the effect of the mycobiome composition on the immunity at specific body sites during skin disease.

Vaginal mycobiota

Like other mucosal surfaces, the vagina is home to a pool of microbial occupants that, if not properly contained, can cause pathologies. While yeast infections are common, several recent studies have revealed a more diverse population of resident fungi than previously appreciated[71-73]. These studies suggest the presence of 11-20 different genera, with

commonly detected fungi including *Candida, Saccharomyces, Aspergillus, Alternaria*, and *Cladosporium*. Changes in fungal diversity were noted to be associated with diabetes, allergic rhinitis and recurrent vaginal candidiasis[72,73]. Fungal growth is controlled both by other members of the local microbiota and by host immune defences. Lactobacilli species are the dominant microorganisms in the healthy vaginal microbiota. These bacteria produce lactic acid that contributes to the low pH of the environment, which suppresses fungal growth, and can compete with *Candida* species for binding to epithelial cells[74-76].

The idea that the immune system is responsible for normal surveillance of the vaginal fungal microbiota is suggested by the association of specific immune gene variants with recurrent vaginal candidiasis. Patients with premature stop mutation in Dectin-1 that is associated with onychomycosis (discussed above) also have recurrent vaginal candidiasis, which suggests that Dectin-1 is important for normal containment of vaginal fungi[60]. Another genetic variant for a length polymorphism in intron 4 of *NLRP3* (encoding NACHT, LRR and PYD domains-containing protein 3) has also been associated with recurrent vaginal candidiasis[77], suggesting that **inflammasomes** are important for containment of vaginal fungi. While an animal model of vaginal candidiasis has not been explored in the context of NLRP3, NLRP3-deficient mice are more susceptible to invasive disease in a model of mucosal *Candida* infection[59]. The group has also reported a role for another inflammasome containing NLRC4 in defence against oral *Candida*, but whether this is important in human defence or in vaginal fungal control is not known[78].

The mannose-binding lectin (MBL) is a soluble C-type lectin receptor that binds to carbohydrates on microbial cell walls where it activates the complement pathway and opsonizes microorganisms for phagocytosis. A genetic polymorphism in MBL that affects the level of protein expression is associated with recurrent vaginal candidiasis, suggesting that MBL is important for normal containment of vaginal fungi (Table 1)[79-81]. The low MBL variant has also been reported to be associated with fungal infections at other mucosal sites such as in defence against *Aspergillus* in the lung[82].

Lung mycobiota

There is little evidence of a commensal fungal microbiota in healthy lungs, although the lungs are constantly exposed to oral and environmental fungi[83]. Thus, the mucosal immune system in the lungs continuously encounters fungi and fungal antigens. When normal lung function is compromised, such as in cystic fibrosis, fungal communities may take hold and persist[84,85]. Commonly encountered fungi in this setting include *Aspergillus* sp. and *Scedosporium* sp., two spore-forming filamentous soil molds that are widespread in the environment. Such spores are inhaled regularly and are normally cleared without pathology in immunocompetent individuals by alveolar macrophages that bind, internalize and kill the spores. Spores that evade killing can grow into hyphae which, if not killed, can invade the tissue, the circulation and disseminate. Disseminated aspergillosis is particularly common in people who lack effective hyphal defence by polymorphonuclear phagocytes. *Aspergillus* sp. is also a potent allergen, and colonization of the airways has been associated with severe asthma and nasal allergies[86].

Recognition of *Aspergillus* sp. in the lungs is mediated in part by pattern recognition receptors including C-type lectins (i.e. Dectin-1 and Dectin-2) and Toll-like receptors. Resting spores are encased in a waxy shell that shields them from recognition by most receptors, but when the spores break this outer coating to germinate and grow as hyphae, multiple cell wall ligands are revealed[87-89]. This germination is generally thought to happen in the lumen of the lung, but Dectin-1 can also recognize *Aspergillus* spores that germinate after ingestion by macrophages in the lung[90]. In this case, the receptor is localized to phagosomes instead of the cell surface and is activated by the presence of the fungus. The central role of alveolar macrophages in initial clearance of inhaled fungi is illustrated in mice expressing the NADPH oxidase only in monocytes and macrophages. These animals clear *Aspergillus* normally, whereas NADPH oxidase-deficient mice are highly susceptible[91,92].

If fungi survive their first encounter with macrophages in the lungs, inflammatory infiltration of immune cells is initiated, and the adaptive immune system may be engaged. Th1 type T cell responses are generally protective against *Aspergillus*, whereas Th2-type responses are generally associated with poor outcomes[93]. However, some reports suggest that Th17 cell responses are even more important than Th1 cell responses. Indeed, it was shown that Dectin-1 signalling specifically is important for decreasing Th1 responses and promoting Th17 cell responses to *Aspergillus*[94]. Also, Dectin-1 is important for stimulating the production of IL-22, a Th17-associated cytokine with pro- and anti-inflammatory properties[95]. Furthermore, Dectin-2 has been shown to have an important role in regulating IL-17 production by a subset of retinoic acid receptor-related orphan receptor-t (RORγt)-expressing neutrophils in response to *Aspergillus*, indicating that these innate cells may be an important source of IL-17 during infection, although this has yet to be specifically examined in the case of exposure to lung fungi[96].

Just as important as mounting effective immunity against fungi in the lungs is the need to prevent allergic inflammation in response to fungal antigens. Allergic responses to *Aspergillus* in a mouse model are highly dependent on Dectin-1 recognition of the fungus and this receptor's ability to promote IL-22 production by T cells[97]. However, the mechanisms promoting allergic inflammation are probably highly dependent on the type of fungus as illustrated by the observation that while *Aspergillus* responses are promoted by Dectin-1, whereas responses to *Cladosporium* sp. were independent of Dectin-1 and IL-17[98]. As noted above, commensal fungi in the gut may have a significant impact on the nature of inflammation in the lungs[26,39]. Exactly how this connection works and whether it preferentially affects responses to fungal triggers are important questions that have yet to be fully explored. It is possible that intestinal fungal-produced PGE2 influences macrophages in the lungs[40-43].

Conclusions

Just as the body is host to diverse populations of bacteria that contribute to health and pathology, so too is the body host to diverse populations of fungi that have been less well studied. Commensal fungal populations vary between body sites and probably vary over time and with disease. It will be interesting in coming years to learn more about how the

immune system specifically interacts with the mycobiota in healthy and disease states. The molecular and cellular mechanisms that engage specific fungi during steady state healthy conditions may be quite different from ones that are engaged in diseased tissue or during active fungal invasion. The health of tissues and tissue barriers might influence this, but so too might the fungi themselves. For example, *Candida* are a dimorphic fungi and can grow in yeast and hyphal forms. This ability to switch between morphologies is important for pathogenesis, and several reports have demonstrated that the yeast and hyphal forms are recognized differently by immune cells[99-104]. A recent study has demonstrated that commensal *C. albicans* in the intestines takes on an entirely novel morphology termed "GUT" that is related to the a/α mating type[105]. GUT *Candida* cells are metabolically adapted to take advantage of nutrients available in the gut and are attenuated in their ability to induce systemic disease in a mouse bloodstream model of infection, but it is not known if they interact with immune cells in ways that are fundamentally different from the other forms.

Each mucosal surface is a unique environment that shapes the microbiota that inhabits the site. Current studies have documented that, like bacteria, diverse populations of fungi are characteristic to specific sites. The local immune system at each site might interact with commensal fungi in similar or unique ways (Figure 3). Future studies will be required to understand how the mucosal immune system interacts with fungal communities and how we might use these interactions to prevent or treat fungal and inflammatory diseases.

Glossary Terms

ASCA	Anti-Saccharomyces cerevisiae antibodies (ASCA) are antibodies that are found commonly in serum from IBD patients. They are more common in Crohn's disease than ulcerative colitis. ASCA cross-react with mannans from cell walls of many fungi (including *Candida* species), suggesting that the name might be misleading.
Candida	*Candida* is a genus of yeasts including common species such as *albicans, tropicalis, glabrata, parapsilosis*, and *krusei. Candida* species are normal inhabitants of the skin and mucous membranes, causing disease primarily in immune compromised people. Candidiasis (disease caused by *Candida*) in the mouth or throat is called "thrush" or "oropharyngeal candidiasis".
CARD9	Caspase recruitment domain-containing protein 9 (CARD9) is a signaling adaptor molecule found to be downstream of many immunoreceptor tyrosine-based activation motif (ITAM) receptors including Dectin-1 and Dectin-2. CARD9 associates with BCL10 and the paracaspase MALT1 to facilitate signaling through NF-κB and promote acute inflammatory responses and initiation of adaptive immunity.
C-type lectin receptor	C-type lectin receptors (CLRs) comprise a large family of receptors that bind to carbohydrates, typically in a calcium-dependent

	manner. Here the term refers to the set of C-type lectin receptors that act as “pattern recognition receptors” in the detection of microbial threats and activate immune responses including membrane receptors such as Dectin-1 & 2, and the mannose receptor, and soluble receptors such as the mannose-binding lectin (MBL). The binding activity of these receptors is mediated by conserved carbohydrate-recognition domains (CRDs).
Chronic mucocutaneous candidiasis	Chronic mucocutaneous candidiasis is a condition characterized by recurrent *Candida* infections of the mouth, skin, and other mucosal surfaces.
Denaturing gradient gel electrophoresis (DGGE)	A DNA-based technique for generating a genetic profile of a microbial community. A highly variable gene (typically 16SrRNA) is targeted for amplification by PCR and the complex mixture of products is separated by denaturing gradient gel electrophoresis which separates fragments based on sequence.
Dysbiosis	A term originally coined by 1908 Nobel laureate Eli Metchnikoff to refer to pathogenic alterations of bacterial microflora in the gut. The term is now generally used to refer to any microbial imbalance in or on the body, including the gastrointestinal tract or the skin, or any exposed mucosal surface such as lungs, vagina, or mouth.
Inflammatory Bowel Disease	Inflammatory Bowel Disease (IBD) is a group of chronic inflammatory conditions of the large and small intestines, the major types being Crohn’s disease and ulcerative colitis.
Inflammasome	Inflammasomes are multimeric protein complexes that activate caspase-1 to promote inflammatory processes such as processing of pro-interleukin-1β and pro-interleukin-18 into their mature secreted forms.
Malassezia	*Malassezia* is a genus of basidiomycetous fungi including species such as *dermatis*, *furfur*, and *restricta*. These yeasts are specifically adapted to growth on mammalian skin. Found on all skin, they are associated with conditions such as dandruff, atopic eczema/ dermatitis, pityriasis versicolor, seborrheic dermatitis, and folliculitis.
Oligonucleotide fingerprinting of rRNA genes (OFRG)	A method for analyzing microbial communities based on hybridization of DNA probes to arrays of cloned DNA fragments representing the ribosomal DNA diversity of the population.
Onychomycosis	fungal infection of the toenails or fingernails. These infections are most commonly caused by dermatophytes (*Microsporum*, *Epidermophyton* and *Trichophyton*), but can also be caused by *Candida* and nondermatophytic molds.

Restriction fragment length polymorphism (RFLP)	A method for characterizing DNA based on sizes of fragments of DNA between restriction enzyme sites. In identification of microbes, “fingerprints” or unique patterns of fragments can be used to distinguish closely-related organisms.
Shotgun sequencing	A sequencing approach in which a complex pool of DNA is broken up into random small segments that are sequenced en masse. Computational tools are then used to reassemble and characterize the DNA fragments.
T helper 1 (T_H1) cell-mediated immunity	a polarization of the T cell response characterized by production of T cells making IFN-γ. This is generally associated with effective host defence against intracellular bacteria and protozoa.
Uncultured Fungi	“Uncultured fungi” is a term used in mycobiome sequencing studies to refer to sequences identified in GenBank as fungal, but of yet-uncharacterized origin.

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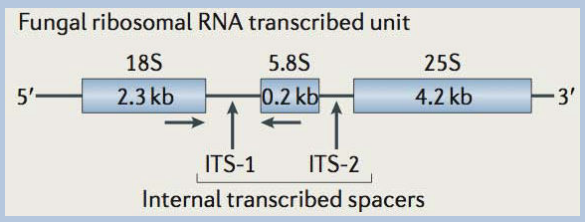
Box 1

Fungal rDNA regions targeted for mycobiome characterization

The genomic region that encodes the fungal ribosomal DNA (rDNA) is shown in the figure. Certain structural regions of the rDNA are highly conserved and permit the synthesis of primers that will amplify fungal rDNA from nearly all species. There is considerable ongoing debate regarding which primer sets are optimal and which induce the least bias into the amplified sequence pool. As in other eukaryotes and bacteria, the rDNA region typically exists in multiple copies in each genome. In fungi, the locus is typically duplicated 100-200 times. As they are not part of the structural ribosomal RNAs that are transcribed from this region, the 'internal transcribed spacer' (ITS) regions are highly divergent between fungi (in sequence and in length), and they are often sufficiently different to classify fungi at the species level. This fungal rDNA region is the most heavily sequenced area in fungal genomes and nearly one-third of publically available fungal sequences are from this region.



Box 2

Sexual dimorphism in fungal taxonomy

Sexual dimorphism in fungi can be a major problem when classifying fungal sequences. Many fungi have a sexual (teleomorph) and an asexual (anamorph) form. Frequently, the two different forms have been given different names and they are often not even characterized as being in the same genus or family. To complicate matters further, many anamorph-teleomorph pairs have not been identified yet and are only being discovered when sequencing reveals that they are the same organism. When relying on public databases to identify fungi based on nucleotide sequences, this can become a considerable challenge. For example, genetically, *Aspergillus glaucus* and *Eurotium herbariorum* are the same organism, although internal transcribed spacer (ITS) sequences that are deposited in the National Center for Biotechnology Information (NCBI) GenBank database are equally split between the two names (see the table for examples). Public databases, including NCBI Taxonomy, can be used to try to resolve some of the known relationships but investigators can often get different taxonomy information depending on which database they use.

Example Anamorph/Teleomorph pairs and the name use in GenBank:

Anamorph	**Teleomorph**
Fusarium graminearum (326 ITS sequences)	*Gibberella zeae* (257 ITS sequences)
Cladosporium herbarum (236 ITS sequences)	*Davidiella tassiana* (177 ITS sequences)
Aspergillus glaucus (16 ITS sequences)	*Eurotium herbariorum* (16 ITS sequences)
Alternaria infectoria (136 ITS sequences)	*Lewia infectoria* (93 ITS sequences)

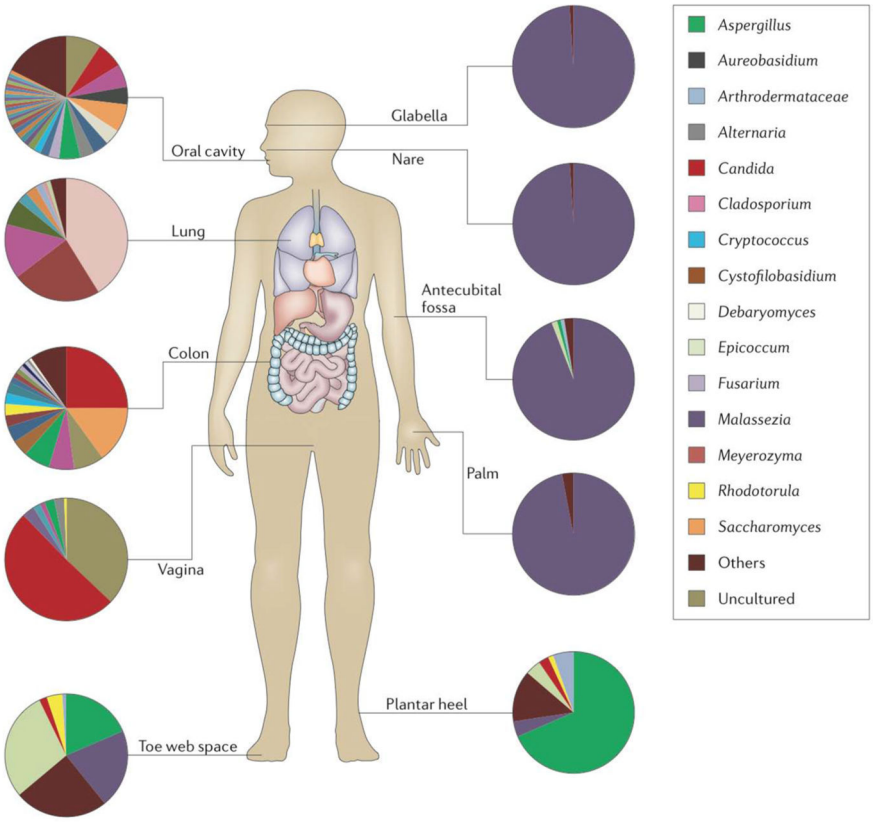


Figure 1. The human mycobiota

Complex populations of fungi have been found associated with all mucosal surfaces and the skin on the healthy human body. The pie charts indicate the relative proportions of fungal genera reported in representative fungal deep sequencing studies. The legend indicates particularly common fungi associated with the respective sites. Mucosal surfaces tend to be more diverse compared to skin. Healthy lung, as in the reported study, likely reflects largely environmental fungi, and are mostly not included in the legend. “Others” in the legend refers to sequences representing less than 1% of the total recovered sequences at each site. “Uncultured” in the legend refers to sequences identified in GenBank as fungal, but of uncharacterized origin. Pie charts were derived from the following studies: Oral[45], Lung[83], Colon[16], Vagina[71], Skin[63].

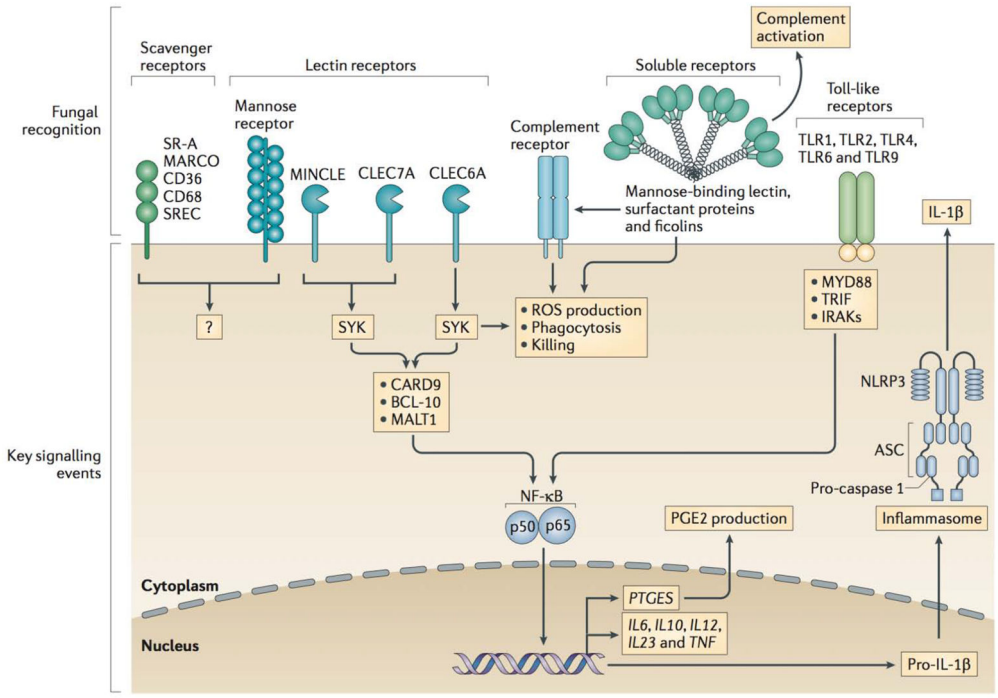


Figure 2. Immune receptors and signalling pathways involved in recognition of fungi

Innate immune cells utilize a wide variety of membrane-bound and soluble receptors to recognize fungi. Membrane-bound receptors such as lectin receptors (that recognize fungal polysaccharides), Toll-like receptors (TLRs), and scavenger receptor family members can directly recognize a wide variety of fungi or soluble products released from fungi. These receptors trigger phagocytosis, respiratory burst via the NADPH phagocyte oxidase, and killing of fungi, as well as trigger intracellular signaling via pathways leading to activation of transcription factors such as NF-κB that mediate production of many inflammatory cytokines and chemokines that are important for host defence against fungi. Fungi may also be recognized by soluble receptors such as the mannose-binding lectin (MBL) that can direct complement activation for killing and release of inflammatory mediators as well as opsonize fungi for recognition by additional membrane-bound receptors such as complement receptors.

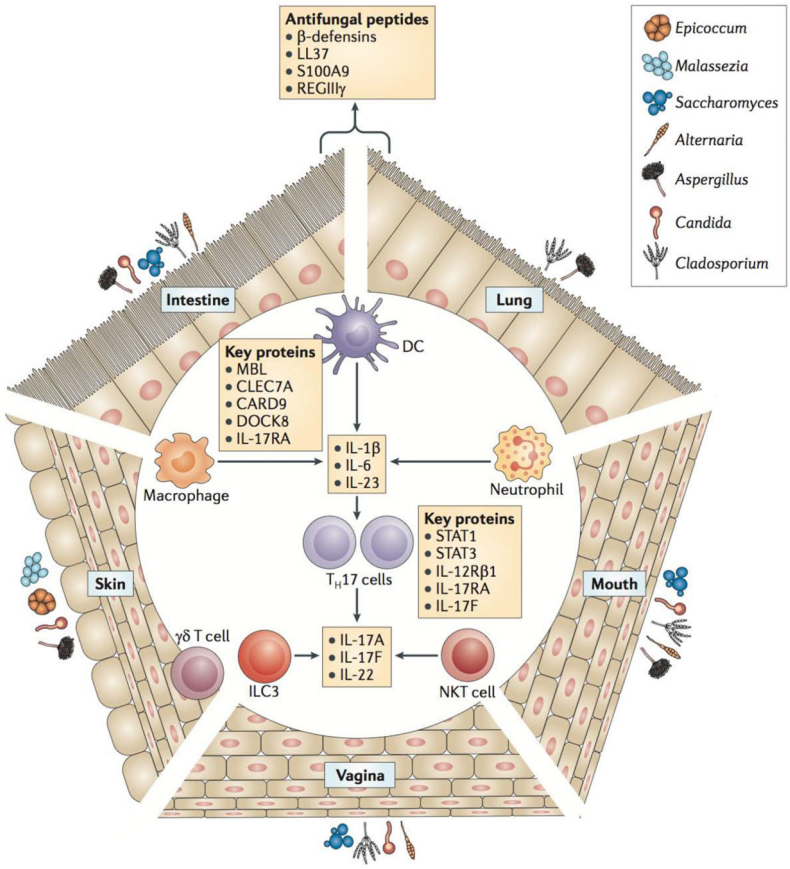


Figure 3. Mucosal immune responses involved in interaction with fungi at different sites

Different body sites are colonized by diverse groups of fungi, and the communities are shaped by the characteristics of each environment. Epithelial cells at these surfaces produce antimicrobial peptides that directly modulate fungal survival. In response to fungi, cytokines and chemokines that recruit immune cells are also produced. Fungi may also be directly sensed by dendritic cells and γδT cells at epithelial surfaces. When epithelial barriers are breached, macrophages, dendritic cells, and neutrophils kill fungi and produce cytokines that promote adaptive immune responses. Innate lymphoid cells may also respond directly to fungi by producing cytokines. Genetic studies in humans have revealed proteins (indicated in red) that are particularly important for antifungal defence and implicate a crucial role for the IL-17 pathway in this process.

Table 1

Genetic disorders associated with impaired mucosal immunity to fungi

Gene	Defects that lead to fungal disease	Disease	Site affected	Mycobiota changes reported	Refs
AIRE	• Autoantibodies against IL-17A, IL-17F and IL-22 • Autoreactive T cells due to loss of tolerance	• Autoimmune polyendocrine syndrome type 1 • APECED • CMC	Skin, nails, oral mucosa and genital mucosa	• *Candida* overgrowth • The effect on the rest of the mycobiota is unknown	59
DOCK8	Defective activation of T cells—including Th17 cells—due to impaired immunological synapse formation	• HIES • CMC	Skin and nails	Increased mycobiota diversity with prevalence of *Candida* and *Aspergillus* species	76, 116, 117
STAT3	• Defective T_H17 cell differentiation • Defective T cell and memory T cell differentiation	• HIES • CMC • Invasive disease	Skin, nails and lungs	• *Aspergillus fumigatus* and *Scedosporium prolificans* lung infection and invasive brain infections • Increased mycobiota diversity with prevalence of *Candida* and *Aspergillus* species	76, 118-121
STAT1	Defective IL-17, IL-22 and IFNγ production	CMC	Skin, nails and oral mucosa	• Candida overgrowth • The effect on the rest of the mycobiota is unknown	53
CLEC7A	Impaired IL-lβ and IL-17 production	• CMC • Onychomycosis	Skin, nails and genital mucosa	• *Candida* overgrowth • The effect on the rest of the mycobiota is unknown	66, 122
CARD9	• Impaired CLEC7A and CLEC6A signalling • Reduced T_H17 cell responses and decreased TNF production	• CMC • Onychomycosis • Deep dermatophytosis • Invasive disease	Skin, nails and genital mucosa	• Candida overgrowth • The effect on the rest of the mycobiota is unknown	46,66
IL17RA	• Impaired responses to IL-1 • Absent IL-6 and GROα production	CMC	Skin and oral mucosa	• *Candida* overgrowth • The effect on the rest of the mycobiota is unknown	57
IL17F	• Impaired production of IL-17	CMC	Skin, nails, oral mucosa and genital mucosa	• Candida overgrowth	91

Gene	Defects that lead to fungal disease	Disease	Site affected	Mycobiota changes reported	Refs
	• Absent IL-6 and GROα production			• The effect on the rest of the mycobiota is unknown	
MBL2	Decreased MBL levels	• Recurrent vulvovaginal candidiasis • Chronic necrotizing pulmonary aspergillosis	Genital mucosa and lungs	• *Candida* or *Aspergillus* overgrowth • The effect on the rest of the mycobiota is unknown	89,91
IL12RB1	Abolished responses to IL-12 and IL-23	CMC	Skin, oral mucosa, oesophageal mucosa and genital mucosa	• *Candida* overgrowth • The effect on the rest of the mycobiota is unknown	123