

Richness and diversity of mammalian fungal communities shape innate and adaptive immunity in health and disease

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Human holobionomes are networks of mutualistic interactions between human cells and complex communities of bacteria and fungi that colonize the human body. The immune system must tolerate colonization with commensal bacteria and fungi but defend against invasion by either organism. Molecular ecological surveys of the human prokaryotic microbiota performed to date have revealed a remarkable degree of bacterial diversity and functionality. However, there is a dearth of information regarding the eukaryotic composition of the microbiota. In this review, we describe the ecology and the human niches of our fungal “fellow travelers” in both health and disease, discriminating between passengers, colonizers, and pathogens based on the interaction of these fungi with the human immune system. We conclude by highlighting the need to reconsider the etiology of many fungal and immune-related diseases in the context of the crosstalk between the human system and its resident microbial communities.

Keywords: Dysbiosis · Fungal metagenomics · Host–microbe interaction · Mycobiota

Introduction

Humans live in close association with a complex community of bacteria, viruses, fungi, and archaea [1–3], which inhabit their bodies. Many groups have surveyed these microbial populations using the so-called “next generation” or “deep” sequencing approaches, revealing that the human microbiota differs radically at various body sites and among individuals [2–4]. The differences in the human microbiota are influenced by the availability of nutrients, environmental exposure to microorganisms, and other site-specific features, such as the immunological makeup of a given location. The origin of differences in the microbiota between individuals potentially reflects different patterns of colonization early in life (reviewed in [5]), different dietary regimens [6, 7], and different environmental exposures, such as antibiotic use [8, 9]. Recently, the application of deep sequencing approaches have gen-

erated novel hypotheses about the microbial determinants underlying human disorders of immune origin (reviewed in [10]). Several metabolites of the interaction between diet and host microbiota, such as short-chain fatty acids, have been shown to play a fundamental role in shaping immune responses (reviewed in [11]). The application of microbial ecology concepts is ultimately leading to the conclusion that health and disease can be understood only through an understanding of the ways in which the symbiotic interactions between microbes and human organs harmonically integrate in the context of the hologenome [12].

Human microbial diversity is not limited to bacteria; microorganisms such as fungi also play major roles in the stability of microbial communities in human health and disease (reviewed in [13]). Yeasts were detected in human stool samples as far back as 1917, and by the mid-20th century the presence of yeasts in the human intestine was proposed to have a saprotrophic role [14]. The mycobiota has been initially studied in animals, ranging from ruminants to insects, such as wasps [15] and termites. These studies paved the way for understanding the role of fungal

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communities in humans. The limited data available thus far suggest that fungal communities are stable across time and are unique to individuals [16, 17]. Even if the available data are fragmentary because it relies mostly on culture-based methods, recent reports using next-generation sequencing technologies also suggest that diverse fungal communities exist in humans [16, 18]. Fungi and *Blastocystis* are the dominant (and in many cases the only) eukaryotes in the gut microbiota of healthy individuals [16, 19]. More diversity will likely emerge when more individuals from diverse populations are sampled using next-generation sequencing, allowing detection of rare taxa. The first culture-independent analysis of the mycobiota populating a mammalian intestine revealed a previously unidentified diversity and abundance of fungal species in the murine gastrointestinal tract [17], indicating that fungi belonging to four major fungal phyla, Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota, account for approximately 2–3% of the total community present in a mucus biofilm. Many culture-dependent studies on various human niches have readily isolated yeasts, such as *Candida* spp., from the mouth, fingernail, toenail, and rectum of healthy hosts [20]. Microbial eukaryotes have also been suggested as the causative agents of diseases such as irritable bowel syndrome, inflammatory bowel disease (IBD), and “leaky gut” syndrome [16, 21, 22]. The primary aim of this review is to describe the fungal communities present in various body sites (Table 1) and the interaction of these fungi with the immune system. Well aware that the number of studies investigating the mycobiota association with humans is small, we reviewed the existing literature describing each fungal species considering its role as commensal, passenger, or colonizer in health and disease. The information summarized in Table 1 is indeed going to rapidly evolve with the exponential increase of community level genome-wide surveys of the microorganisms inhabiting the various microenvironments of the human body (i.e., gut, skin, oral mucosa, and urogenital tract) [23], their environmental reservoir [24], and the human populations living in different geographic regions [6, 8].

Understanding the prevalence and distribution of microbial eukaryotes in addition to prokaryotic microorganisms in the human body may have important consequences for human health. While current studies of the human mycobiota focus mainly on pathogens or opportunistic fungi, most resident microbial eukaryotes do not cause infections, and are instead either beneficial or commensal. Elucidating community-wide changes in the human mycobiota, rather than only the presence or absence of specific taxa, will be crucial to understanding the cause of, and potential treatment for, several multifaceted polymicrobial diseases [25].

Immune response to fungi: Balance between tolerance and pathogenicity

Immune responses to fungi require PRRs, such as TLRs, C-type lectin receptors, and the galectin family of proteins [26–28] to trigger intracellular signaling cascades that initiate and direct

innate and adaptive immune responses [29]. By sensing conserved molecular structures on fungi, namely the PAMPs, PRRs promote the activation of the immune system and the clearance of fungi, with specific immune responses generated depending on the cell type involved. In a recent review [30], we highlighted the roles and mechanisms of dectin-1, dectin-2, and DC-SIGN in orchestrating antifungal immunity, exploring how these PRRs help maintain homeostasis between potential disease-causing organisms and resident microbial populations. Indeed, the immune system does not remain ignorant of commensal, passenger (transient), or opportunistic fungi, and sensing these different fungi through PRRs serve to ensure that both the symbiotic host–microbial relationship and a homeostatic balance between tolerogenic and proinflammatory immune responses are maintained. In light of this, tissue homeostasis and its possible breakdown in fungal infections and diseases play a fundamental role.

A number of seminal reviews have addressed the importance of both resistance — the ability to limit microbial burden — and tolerance — the ability to limit the host damage caused by an uncontrolled response — as mechanisms of immune responses to fungi and the reader is directed to these for more in-depth information about specific immune mechanisms [31–34].

Monocytes, macrophages, neutrophils as well as epithelial and endothelial cells [35], mostly contribute to the antifungal innate immune response through phagocytosis and direct pathogen killing. By contrast, uptake of fungi by DCs promotes the differentiation of naïve T cells into effector Th-cell subtypes (Fig. 1). A healthy interaction between fungi and the host requires the interplay of several Ag-specific adaptive immune responses, the first step being the activation of the innate fungal detection system through the PRR.

In tissues, inflammatory signals mediated by direct recognition of fungal cell wall components or other fungal products by PRRs, recruit additional immune cells and drive adaptive immune responses. IFN- γ produced by Th1 lymphocytes is fundamental for stimulating the antifungal activity of neutrophils. The central role of endogenous IFN- γ in the resistance against systemic fungal infection is underscored by the observation that KO mice deficient in IFN- γ are highly susceptible to disseminated *C. albicans* infection [36]. In addition, mice deficient in IL-18, which plays a crucial role in the induction of IFN- γ , are also more susceptible to disseminated candidiasis [37]. Th1 also appears to be protective in the host defense against *Aspergillus*. Cells producing IFN- γ are induced by *Aspergillus* in immunocompetent mice. Live conidia, which undergo swelling and germination, are able to prime Th1 responses [38]. It has been elegantly demonstrated that CD4⁺ T cells differentiate during respiratory fungal infection, with TLR-mediated signals in the lymph node enhancing the potential for IFN- γ production, whereas other signals promote Th1 differentiation in the lung [39]. Although many studies focused on the pathological aspects of IL-17-producing T cells in many autoimmune diseases, studies examining T-cell polarization in response to PAMPs have identified an array of fungal components that preferentially induce the Th17 lineage [40], suggesting a role for Th17 cells in fungus-induced host defense, such as those

Table 1. Different fungal genera dominate different body sites

Fungus	Body site	Role	Presence in health and disease
<i>Aspergillus</i>	Skin	Opportunistic fungus	Presence increased in PID patients [79]
	Mouth	Opportunistic fungus	Present in healthy mycobiota but increased in immunocompromised patients [82, 111, 112, 114]
	Lung	Opportunistic fungus/pathogen	Present in both status but dominant in transplant patients [122] and CF patients [139, 140] and known causative agent of ABPA and asthma
<i>Aureobasidium</i>	Mouth	Commensal/transient	Present in health [82]
<i>Blastocystis</i>	Gut	Commensal	Dominant in health [19]
<i>Candida</i> sp. (in particular <i>C. albicans</i>)	Skin	Commensal	Dominant in AD patients [150]
	Mouth	Commensal/opportunistic fungus	Present in health [82], dominant in immunocompromised patients [110, 114]
	Lung	Commensal/opportunistic fungus	Present in healthy mycobiota [82], dominant in transplant patients [122] and in CF [138, 140]
	Gut	Commensal/opportunistic fungus	Present in health and disease [19, 151]
<i>Cladosporium</i> sp.	Skin	Transient during health/colonizer in disease	Present in AD patients but seldom detected in healthy samples [104]
	Mouth	Commensal	[82]
	Lung	Opportunistic fungus	Present in transplant patients [122] and contributing to asthma
<i>Cryptococcus</i> sp. (in particular <i>C. diffluens</i> , <i>C. liquefaciens</i>)	Skin	Commensal	Dominant in healthy mycobiota [91, 106], decreased in dandruff-affected individuals [106].
	Mouth	Commensal	Detected as part of healthy mycobiota [82]
	Lung	Commensal in health, colonizer, and opportunistic pathogen	Present in health and disease but dominant in transplant patients [122]
<i>Debaromyces</i> sp.	Skin	Commensal	Present in health [103]
<i>Filobasidium</i>	Skin	Opportunistic fungus	Increased in dandruff-affected individuals [106]
<i>Fusarium</i>	Mouth	Commensal	[82]
<i>Geotrichum</i>	Mouth	Opportunistic fungus	Detected in immunocompromised patients [82, 111, 114]
<i>Hemispora</i>	Mouth	Opportunistic fungus	Detected in immunocompromised patients [82, 111, 114]
<i>Hormodendrum</i>	Mouth	Opportunistic fungus	Detected in immunocompromised patients [82, 111, 114]
<i>Malassezia</i> sp.	Skin	Commensal/opportunistic fungus	Dominant in healthy mycobiota, associated with several skin diseases such as AD; dominant in dandruff-affected individuals [116], decreased in PID patients [79]
	Mouth	Commensal/opportunistic fungus	Present in healthy subjects [82]
<i>Penicillium</i>	Lung	Opportunistic fungus	Increased in CF [138]
	Mouth	Opportunistic fungus	Increased in immunocompromised patients [82, 111, 112]
	Lung	Opportunistic fungus	Present in health but dominant in CF patients [138] and asthma
<i>Pichia</i>	Mouth	Commensal/resident	Abundant in healthy subjects, decreased during <i>Candida</i> infection [114]
<i>Pneumocystis</i>	Lung	Commensal/opportunistic	Present at low amount in healthy, causing disease in immunocompromised hosts [114] and CF patients [125]
<i>Rhodotorula</i> sp.	Skin	Commensal	Present in healthy subjects [103]
<i>Saccharomycetales</i> <i>S. cerevisiae</i>	Mouth	Commensal/transient	Present in health mycobiota [82], dominant in immunocompromised patients [110]
	Gut	Commensal/transient	Detected in health mycobiota [19, 80, 149] and during CD (De Filippo et al., submitted)
<i>Scopulariopsis</i>	Mouth	Opportunistic fungus	Detected in immunocompromised patients [72, 111, 112]
<i>Toxicocladosporium irritans</i>	Skin	Opportunistic fungus	Detected in AD patients [104]

The presence in health and disease of the main fungal genera, inhabiting or transiently in contact with the human body, is indicated. AD: atopic dermatitis; PID: primary immunodeficiency; ABPA: allergic bronchopulmonary aspergillosis.

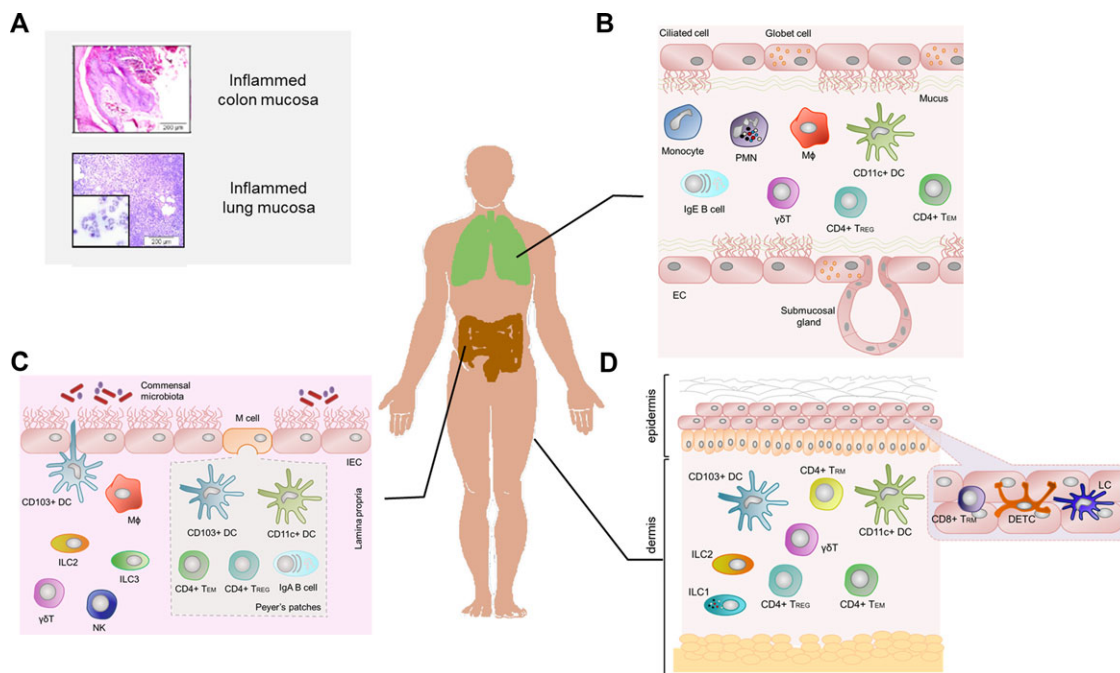


Figure 1. Immunological players in the crosstalk between mucosal immunity and resident mycobiota. The major cells participating in the immune responses in various body sites are depicted along the human body. (A) The lung and colon mucosa after infection of mice with *A. fumigatus* and *C. albicans*, respectively. Paraffin-embedded tissue sections (3–4 μm) were stained with periodic acid-Schiff reagent and monocytes infiltration at the site of infections highlighted. Photographs were taken with an Olympus BX51 microscope at 10 or 40 \times (insets). (B) Lung cellular community. Ciliated cells sweep the mucus, which protects the luminal zone by transporting antimicrobial peptides, out of the lung. When the fungus breaks the airway epithelium, released chemokines recruit circulating neutrophils and macrophages. DCs sample the airway lumen by forming dendritic extensions between epithelial cells. DCs form tight junctions with epithelial cells through their expression of occludin and claudin family members. Their activation in turn prime the Th-mediated immune response. When allergens are sensed, DCs activate Th2-cell and B-cell responses, with IgE production. (C) The mucosal barrier of the gut. Ags contact specialized microfold (M) cells overlying Peyer's patches, which then process and present the Ag to the immune system. Here, CX3CR1⁺ macrophages and CD103⁺ DCs collaborate in capturing soluble food Ags promoting oral tolerance by activation of T-cell and B-cell responses. When B lymphocytes are stimulated by antigenic material, the cells develop into Ab-forming cells that secrete various types of Igs, the most important of which is IgA. (D) Skin cellular community. Epidermis is interspersed with three important immune cell types, Langerhans cells, $\gamma\delta$ T cells (termed DETCs) and CD8⁺ TRM cells representing the first line of defense in skin immunity. The dermis contains cells of the immune system including Treg cells, CD4⁺ T effector memory cells (TEM cells), other $\gamma\delta$ T cells, ILCs, and several populations of DCs. Adaptive T-cell response is controlled by resident Treg cells. M ϕ , macrophage; LC, Langerhans cell; DETC, dendritic epidermal T cell.

specific for *C. albicans*, *Pneumocystis carinii*, and *Cryptococcus* spp. The observation that mice deficient in IL-17RA show an increased susceptibility to disseminated *C. albicans* infection first demonstrated the critical involvement of Th17 responses in protective anti-*Candida* host defenses [41]. Although this suggests a protective role for Th17 response in fungal infection, negative effects of Th17-mediated inflammatory responses to intragastric *C. albicans* infection in mice have also been reported [42], as well as higher susceptibility to *Candida* and *Aspergillus* infection in absence of Toll IL1R8 (TIR8), a negative regulator of Th17 responses [43]. On the other hand, patients with impaired *Candida*-specific Th17 responses, such as patients with chronic mucocutaneous candidiasis, are especially susceptible to mucosal *C. albicans* infections [44]. These observations strongly indicate that Th17 responses are important for human anti-*Candida* mucosal host defense since patients with genetic defects in the receptor dectin-1 or in its signaling (a potent activator of Th17) suffer from chronic mucosal fungal infections [45, 46]. Mucosal Th17-cell subsets and their associated cytokines, IL-17A, IL-17F, and IL-22, have been shown

to play key roles in discriminating colonization and invasive fungal disease [47–49].

Apart from its protective role, the Th17 response to fungi can be reciprocally regulated by the Th1, Th2, and regulatory branches of adaptive immunity and vice versa [50–52]. Both IL-23 and IL-17 have been shown to impair the antifungal effector activities of mice neutrophils by counteracting the IFN- γ -dependent activation of IDO (see below), which is known to limit the inflammatory status of neutrophils against fungi, such as *A. fumigatus* [53], and which likely accounts for the high inflammatory pathology and tissue destruction associated with Th17-cell activation. In its ability to inhibit Th1 activation, the Th17-dependent pathway could be responsible for the failure to resolve an infection in the face of ongoing inflammation. IL-17 neutralization was shown to increase *A. fumigatus* clearance, ameliorate inflammatory pathology murine lungs, and restore protective Th1 antifungal resistance [54].

The complex fungal communities encompassing food-borne and environmental fungi present in the host dictate the

generation of the different Th-cell subtypes as a result of exposure to different microbial adjuvants. For example, fungal β -glucan mediated dectin-1 activation on the surface of human DCs induces CD4⁺ Th1- and Th17-cell proliferation [55] and primes cytotoxic T cells in vivo [56]. Other fungal cell wall Ags, such as chitin, have been shown to alternatively activate macrophages to drive Th2 immunity [57].

However PRRs might be used by fungi to escape and subvert the host immune responses in order to survive and eventually replicate, that is, the *C. albicans* induction of IL-10 release through TLR2 [58]. The ability to switch between yeast and hyphal growth is one of the key virulence attributes of *C. albicans*: this causes the blockade of TLR recognition by Ag modification during the germination of yeasts into hyphae [59]. It is clear that yeast and hyphae induce different responses [60] by exposing different cell wall Ags [61] to protective immunity.

Thus, the nature of cell wall Ags likely also serves to promote a specific inflammatory phenotype. Indeed, fungal pathogenicity should be examined in the context of features of host responses to environmental and commensal fungi and the circumstances that influence the balance between healthy, tolerated exposure to fungi, and pathogenicity, seen as a loss of balance of the resident microbial communities and their relative abundance in different bodily sites and organs.

Fungi as immunoregulators

Commensal microbes significantly shape mammalian immunity, both at the host mucosal surface and systemically [62, 63], controlling unexpected microbial burden and growth. However, it is unclear how opportunistic fungi, such as *C. albicans*, remain at mucosal surfaces in the face of adaptive immunity as commensals, that is, as components of the mycobiota of a healthy host. Here, the fungus is controlled by (i) the microbial flora of the healthy host, (ii) the epithelium, which is able to secrete antimicrobial peptides, and (iii) the local innate immune system. *Candida* spp. can clearly grow in the intestines, coexist with the bacterial microbiota, bloom during antibiotic perturbations of the microbiota, and colonize inflamed mucosa, leading to life-threatening infections. Although the commensal stage is frequently described as “harmless” to the host, it is likely that this stage is highly regulated and the fungus is continuously or transiently interacting with the host immune system [64]. It is also likely that the human host has evolved to recognize and deal with a potential fungal invader so that the evolved state is one of commensalism. Treg cells seem to act in tuning this equilibrium by preventing inappropriate immune responses that can be damaging to host tissues. Bacher et al. [65] found that Treg cells specific for *A. fumigatus* and *C. albicans* — both of which inhabit or are in contact with our mucosae — exceeded in number and functionally suppressed specific memory T cells. In patients with severe allergic reactions, the Ag-specific memory T-cell response dominated the immune environment [65]. Thus, expansion of fungus-specific Treg-cell populations is important for preventing pathological immune responses.

Indeed, while early inflammation prevents or limits infection, an uncontrolled response may eventually oppose disease eradication. Counteracting exaggerated effector immune responses and dysregulated inflammation requires a specific environment in which not only Treg cells but also tolerogenic DCs play an essential role. The shift between the inflammatory and anti-inflammatory states of DCs is strictly controlled by the kynurenine pathway of tryptophan catabolism, and it has been shown to involve IDO [66]. IDO activates the aryl hydrocarbon receptor (AhR) in lymphoid tissues [67] and promotes Treg-cell development [68]. In the gastrointestinal (GI) tract, diet-derived AhR ligands promote local IL-22 production by innate lymphoid type 3 cells (ILC3s) [69]. In combination with IL-17A, IL-22 mediates a pivotal innate antifungal resistance in mice [70] and humans [71]. Zelante et al. [72] showed that the intestinal microbiota regulates these cytokines, and in particular a subset of commensal *Lactobacilli* — *L. reuteri* in the stomach and *L. acidophilus* in the vaginal tract — produce the metabolite indole-3-aldehyde by tryptophan metabolism, and indole-3-aldehyde activates AhR in ILCs. This AhR activation results in an induced IL-22-mediated antimicrobial response, which in turn reduces colonization by opportunistic fungi, such as *Candida*, providing mucosal protection from inflammation.

In the complex host–pathogen interaction used by both parties to evaluate the environmental milieu in the ongoing battle for survival, *Candida* in turn has been shown to produce immunomodulatory compounds, such as oxylipins from the conversion of polyunsaturated fatty acids [73]. Those molecules interfere with the metabolism, perception, and signaling processes of cell immune response.

In parallel, mucosal fungi modulate both the innate and adaptive immune responses. In immunocompetent mice, it was shown that while two consecutive airway exposures to *A. fumigatus* conidia stimulate neutrophil and macrophage recruitment to the lung and prime a Th1 response to the fungus, repeated exposures to *A. fumigatus* conidia does not result in invasive aspergillosis or fatal disease, but does result in the development of chronic pulmonary inflammation [74] mediated by Th2 and Th17 responses. Therefore, it is likely that repeated pulmonary exposure to *A. fumigatus* conidia eventually leads to immune homeostasis and the induction of non-T-cell regulatory pathways that result in the least possible tissue damage while still controlling conidial germination [75, 76].

Candida albicans has been shown to have the capacity to “train” innate immunity toward other microorganisms, such as intestinal and skin bacteria [77–79]. Furthermore, *Saccharomyces cerevisiae*, previously considered a transient microorganism in the intestinal tract, has been increasingly reported to be present in the human skin as well [17, 80–82]. We recently observed that the presence of *S. cerevisiae* among the gut microbiota “educates” the host immune response by means of training the immune system to better cope with a secondary infection (Rizzetto et al., unpublished and De Filippo et al., unpublished).

The immunomodulatory role of commensal organisms has been formalized by the “hygiene hypothesis,” which suggests that reduced early exposure to microorganisms is the main cause of the

early onset of autoimmune or chronic inflammatory disorders in the industrialized world [83]. Several microorganisms, including some *Clostridium* spp., have been shown to drive immunoregulation and to block or treat allergic and autoimmune disease and IBD [84–86]. The immunoregulatory mechanisms used by several bacteria, such as *Bacteroides fragilis*, *Clostridium* [84], or by helminths [85] are based on the specific induction of Treg cells in the colon or skin, or by the induction of regulatory DCs [87]. We speculate that an overall reduction in early exposure of humans to beneficial microbiota is not simply causing a reduction in anti-inflammatory signals but is more importantly decreasing the “training” of our immune system to handle pathogenic microorganisms, possibly resulting in uncontrolled immune responses. Collectively, these findings show that eukaryotic and prokaryotic communities are kept in equilibrium by mutual interactions that include the production of immune modulating molecules, helping to accommodate fungi, either commensals or ubiquitous, within the immune homeostasis and its dysregulation.

Fungi inhabiting the cutaneous mucosal surface: The skin mycobiota

The skin represents the primary interface between the human host and the environment. Cutaneous inflammatory disorders such as psoriasis, atopic dermatitis (AD), and rosacea have been associated with dysbiosis in the cutaneous microbiota [88, 89]. Recent metagenomic studies, investigating the bacterial and fungal components of the human skin microbiota of healthy subjects, have uncovered surprising differences in microbial diversity between distinct topographical skin sites [81] (Table 1). For example, 11 body sites, including the arm, were found to be dominated by *Malassezia* fungi (Table 1), but in contrast, other sites including the foot (plantar heel, toenail, and toe web) exhibited a broad fungal diversity, with the presence of a wide range of fungal genera (i.e., *Rhodotorula*, *Debaromyces*, *Cryptococcus*, and *Candida*) [90]. These results demonstrate that physiologic attributes and topography of the skin differentially shape the mycobiota and microbiota composition.

Indeed a healthy microbiota benefits many aspects of skin physiology, including wound healing [91], protection against pathogens [92], and normal development of immune responses in the skin [77]. Members of the microbiota that dwell deep in the dermis layers and hair follicles help in recolonization of the epidermis, hair follicles, and sebaceous glands, and produce molecules that exert immunoregulatory effects [93]. For instance, after wounding, staphylococcal lipoteichoic acid has been shown to inhibit both inflammatory cytokine release and inflammation triggered by injury through a TLR2-dependent mechanism [92]. In this complex homeostasis, keratinocytes, which express many PRRs, such as the TLR family [94], have a key role in the innate immune response against pathogens, contributing to both detection and defense [95]. In direct response to microbes, or through indirect activation by cytokines such as IL-22, keratinocytes can produce a wide array of antimicrobial peptides,

such as beta-defensin and the cathelicidin LL-37 [96, 97]. Several other immunological players with various roles in skin immunity have been detected in the epidermis and dermis (Fig. 1) (for a review see [98]), such as migratory CD8⁺ DCs [99]. Langerhans cells (LCs) contribute to priming adaptive T-cell immunity to skin pathogens such as yeast (*C. albicans*) and bacteria (*Staphylococcus aureus*), favoring the induction of Th17-cell responses by direct Ag presentation to Th17 cells [100]. LCs also appear to be immunosuppressive, either through inducing T-cell deletion or activating Treg cells that dampen skin responses to fungi [101]. Using a BM chimeric mouse model in which only LCs express MHC class II IE, it was demonstrated that CD4⁺ T cells responding to Ag presentation by activated LCs initially proliferated but then failed to differentiate into effector/memory cells or to survive long term [101]. The tolerogenic function of LCs was maintained after exposure to potent adjuvants consistently with their failure to translocate the NF- κ B family member RelB from the cytoplasm to the nucleus [101]. This commitment of LCs to tolerogenic function may explain why commensal microorganisms confined to the skin epithelium are tolerated, whereas invading pathogens that breach the epithelium and activate dermal DCs stimulate a strong immune response.

In the presence of *C. albicans*, LCs have been shown to stimulate both skin-resident Treg-cell and effector T-cell proliferation [102]. This specific induction has been demonstrated to be mediated by Ag presentation mechanism — via CD80/86, HLA-DR — and IL-15 pathways [102]. Together, the above findings support a model in which LCs provide important regulatory feedback to the immune system, but also selectively contribute to effector T-cell responses. Resident commensal organisms on the skin are necessary for optimal cutaneous immunity, through the increase of IL-1 β signaling and amplifying responses in accordance with the local inflammatory environment [85]. Screening mice deficient in factors known to drive IL-17A production, Hanski et al. showed that IL-1R1, and its downstream signaling complex MyD88, play a dominant role in controlling the production of IL-17A, but not IFN- γ , by cutaneous T cells. IL-1 α production by cutaneous cells was significantly reduced in germ-free mice and monoassociation with *S. epidermidis* restored the production of this cytokine, showing that resident bacteria are necessary to drive effector T-cell function in the skin [85]. The skin can be a point of entry for fungal infections when the epithelial barrier is breached, or it can be a site for disseminated, systemic fungal diseases. For example, the dryness associated with AD compromises the barrier function of the skin and as a result AD is associated with high susceptibility to viral, bacterial, and fungal skin infections [103]. To determine whether the skin microbiota of patients with AD is different from that of healthy individuals, Zhang and co-workers used an rRNA gene clone library of 3647 clones to identify 58 fungal species and seven unknown phylotypes from AD patients and healthy individuals [104]. As expected, *Malassezia* species were predominant in AD patient skin, accounting for 63–86% of the clones identified from each subject. Overall, the non-*Malassezia* yeast microbiota of the patients was more diverse than that of the healthy subjects. *Candida albicans*, *C. diffluens*, and *C. liquefaciens* as well as the

filamentous fungi *Cladosporium* spp. and *Toxicocladosporium irritans* were detected in AD samples but were seldom detected in healthy samples [104]. Although *Malassezia* yeasts are a part of the mycobiota of healthy skin, they have also been associated with a number of diseases affecting the human skin, such as pityriasis versicolor, folliculitis, seborrhoeic dermatitis and dandruff, psoriasis, and AD (for a review see [105]). Changes in the fungal microbiota of the scalp that accompany dandruff have been examined [106]. While fungi of the Ascomycota dominated in both healthy individuals and dandruff patients, fungi of the Basidiomycota phyla (which include *Malassezia*) were significantly increased in dandruff-afflicted scalps [106]. Specific genera that were increased in patients with severe dandruff included *Filobasidium*, which jumped to 94% of the total basiomycetes — completely replacing the *Cryptococcus* spp. presents in healthy subjects, and *Malassezia* (5%) — which represents a twofold increase over healthy samples. In addition to the basiomycete fungi of the genus *Cryptococcus*, healthy scalps were dominated by *Acremonium* spp. and *Didymella bryoniae* (over 95% of the Ascomycota) [106].

An exemplary recent publication [79] has added further fundamental understanding of the role of skin microbiota in activating and educating host immunity, shedding new light on the interplay between the immune system and microbiota. The authors studied patients with hyper IgE syndrome, a primary immunodeficiency resulting from STAT3 deficiency, and compared the bacterial and fungal skin microbiota at four clinically relevant sites representing the major skin microenvironments (the nares, retroauricular crease, antecubital fossa, and volar forearm) [79]. The patients displayed increased ecological permissiveness, characterized by altered microbial population structures including colonization with bacterial microbial species not observed in healthy individuals, such as *Clostridium* species and *Serratia marcescens* [79]. An elevated fungal diversity and increased representation of opportunistic fungi (*Candida* and *Aspergillus*) were observed in hyper IgE syndrome patients, concomitant with a decrease in the relative abundance of the common skin fungus *Malassezia* [79]. These changes supported the hypothesis of increased skin permissiveness to microbial transit, suggesting that skin may serve as a reservoir for the recurrent fungal infections observed in these patients [79].

The differences in the cutaneous microbiota between healthy individuals and primary immunodeficiency patients probably correlate with their immunological status. Defects in STAT3 signaling impair defensin expression and the generation and recruitment of neutrophils [107], in part due to defects in Th17-cell differentiation. These findings further suggest that altered immune responses in disease modify not only the bacterial microbiota niche but also the fungal skin/mucosal communities, which may contribute to the increased fungal infections observed clinically in this patient population. The skin microbiota investigation provides an important step toward understanding the interactions between pathogenic and commensal fungal and bacterial communities, and how these interactions can result in beneficial or detrimental (i.e., disease) outcomes. Species often considered “normal” colonizers of the skin, such as *Malassezia*, can

become causal agents of skin diseases. These preliminary results indicate the difficulty of defining a “normal” microbiota and consequently, meaningfully linking the mycobiota with clinical status would require a significant increase in the number of samples analyzed.

Fungi inhabiting the mouth: The oral mycobiota

The oral microbiota is a critical component of health and disease. Disruption of this community has been proposed to trigger or influence the course of oral diseases such as periodontal disease and oral mucositis, especially among immunocompromised patients such as those undergoing chemotherapy and HIV patients (reviewed in [108]). Metagenomic sampling of individual sites within the oral cavity shows that there are probably hundreds of different microbial niches in the human mouth [58, 59]. The fungal component of the oral microbiota, however, has been only recently characterized.

Ghannoum et al. performed the most comprehensive study to date on the fungal microbiota of the mouth by using a multitag pyrosequencing approach, combined with the use of pan-fungal internal transcribed spacer (ITS) primers [82]. The authors found that the distribution of fungal species in the mouth varied greatly between different individuals. The mycobiota of a healthy human mouth encompasses 74 cultivable and 11 non-cultivable fungal genera [82]. The core fungal mycobiota comprises *Candida* species (the most frequent, isolated from 75% of participants), *Cladosporium* (65%), *Aureobasidium* (50%), *Saccharomycetales* (50%), *Aspergillus* (35%), *Fusarium* (30%), and *Cryptococcus* (20%) [82]. Four of these main genera, namely *Aspergillus*, *Fusarium*, *Cryptococcus*, and *Cladosporium*, are known human pathogens: the impact of their presence as a warning signal of increased risk of infection needs to be addressed. The remaining 60 nonpathogenic fungi detected in the oral wash samples represent species that likely originate from the environment in the form of spores inhaled from the air, or from material ingested with food. Thus, the presence of these microbes in the oral cavities of healthy individuals was not necessarily surprising, but the observation that transient colonization by environmental fungi may occur in the oral cavity (and upper airways) has potential implications for hypersensitivity diseases.

Recently, Dupuy et al. detected *Malassezia* spp. in the saliva of healthy subjects using high-throughput sequencing analysis of ITS1 amplicons [109]. As already described, *Malassezia* spp. are dominant, highly adapted commensals/pathogens (i.e., their pathogenic potential is unleashed upon failure from the immune system to keep them at bay) of human skin, suggesting a potential additional importance of these organisms in the core mycobiota of the healthy human mouth.

The presence of pathogenic fungal isolates in the oral cavity of healthy individuals is quite unexpected and the clinical relevance is unknown. It is possible that the presence of a given fungal isolate in an individual could be the first step toward predisposing that individual to opportunistic infections. The

pathogenicity of the fungi in the oral environment may be controlled in healthy individuals by other fungi or other member of the oral community, as well as by the functional immune system, suggesting that interdependent crosstalk may exist between constituents of the oral mycobiota. Surveying 18S rDNA using a PCR-based approach, Aas et al. [110] reported the presence of *C. albicans* and *S. cerevisiae* in the subgingival plaque microbiota of HIV-infected patients. Other fungi previously found in the oral cavity of immunocompromised patients include *Penicillium*, *Geotrichum*, *Aspergillus*, *Scopulariopsis*, *Hemispora*, and *Hormodendrum* [89, 111, 112], although the representation of species seems to correlate with the geographic area of sampling [113]. Recently, Mukherjee et al. used pyrosequencing to characterize the oral microbiota of 12 HIV-infected patients and 12 healthy subjects [114]. The core oral bacterial microbiota comprised 14 genera, of which 13 were common between patients and healthy subjects. In contrast, the core oral mycobiota differed more between HIV-infected and -uninfected individuals, with *Candida* being the predominant species in immunocompromised patients (98 versus 58% in healthy subjects). Among HIV-infected patients, *Candida*, *Epicoccum*, and *Alternaria* were the most common genera, while in uninfected participants, the most abundant fungi were *Candida*, *Pichia*, and *Fusarium*. Increase in *Candida* colonization, particularly that of *C. albicans*, was associated with a concomitant decrease in the abundance of *Pichia* — a resident oral fungus representing the 33% of healthy oral mycobiota, suggesting an antagonistic relationship. Indeed, *Pichia* has been shown to inhibit growth of pathogenic fungi such as *Aspergillus* and *Candida* by inhibiting the ability of these genera to adhere, germinate, and form biofilms in vitro [114].

Oral *Candida* colonization is a known risk factor for invasive candidiasis [115]. Similarly, fungal caused periodontal disease is associated with rheumatoid arthritis [116] and atherosclerosis [117], suggesting that bacterial and fungal microbiota from the oral cavity may contribute to the development of certain human diseases.

Fungi inhabiting the respiratory tract: The lung mycobiota

The human respiratory tract represents the major entry point for numerous microorganisms, primarily airborne viruses, bacteria, and fungal spores. Certain characteristics of these microorganisms, such as *Aspergillus* spp., coupled with the local host immune response, determine whether the microorganisms will be cleared by the immune system or adhere to and colonize the airways, leading to acute or chronic pulmonary disease [118].

The lower respiratory tract (trachea, bronchi, and pulmonary tissue), previously thought to be sterile when healthy, has recently been shown to clearly harbor a low level bacterial microbiota, which changes during disease (reviewed in [119]). Any microbe, be it a bacterium or a fungus, reaching the lower respiratory tract encounters the efficient cleansing action of the ciliated epithelium.

Microorganisms are also subsequently removed by coughing, sneezing, and swallowing. However, if the respiratory tract epithelium becomes damaged, as in the case of bronchitis or viral pneumonia, the individual may become susceptible to infection by pathogens descending from the nasopharynx (upper respiratory tract). The bacterial component of the microbiota of a healthy host has been addressed more deeply than its fungal counterpart. The bacterial microbiota consists of nine core genera: *Prevotella*, *Sphingomonas*, *Pseudomonas*, *Acinetobacter*, *Fusobacterium*, *Megasphaera*, *Veillonella*, *Staphylococcus*, and *Streptococcus* [120, 121] but little data exist about the fungal microbiota of the lungs, with the exception of *Pneumocystis* spp. In a recent study by Charlson et al., the fungal microbiota of the mouth and lungs in select healthy and lung transplant recipients was analyzed by ITS-based pyrosequencing [122]. The fungal distribution in the oral wash of healthy subjects was similar to that found in the study by Ghannoum et al. [82]. In the lung transplant recipients, the fungal microbiota of the oral cavity was dominated by *Candida*, likely depending on the antibiotic and immunosuppressant used by these patients [122]. The bronchoalveolar lavage from lung transplant recipients showed detectable *Candida* spp., *Aspergillus* spp., or *Cryptococcus* spp. Because all of the transplant recipients had been treated with antibiotics and immunosuppressants, thus ablating host immune responses and the prokaryotic milieu of the lung microbiota, this first study supports the notion that host defense, and perhaps some sort of bacterial microbiota-mediated resistance mechanisms, play a major role in keeping fungal colonization extremely low in the lungs.

Numerous studies have indicated that Th17 cells and their signature cytokine IL-17A are critical to the airway's immune response against various infections, including intracellular bacteria [123, 124] and fungi [125]. The innate IL-17A-producing cells, $\gamma\delta$ T cells have been shown to act on nonimmune lung cells in infected tissues to strengthen innate immunity by inducing the expression of antimicrobial proteins and inflammatory chemokines as CCL28, in those cells, causing the migration of IgE-secreting B cells to the infected tissues [126] as well as the proliferation of human airway epithelial cells in vitro. Additionally, IL-17A production by pulmonary $\gamma\delta$ T cells in the early phase of tuberculosis infection stimulates neutrophil recruitment to the infected tissues [127, 128].

Neutrophils release their genomic DNA into the extracellular environment in the form of neutrophil extracellular traps (NETs) [129] and ensnare invading pathogens [130, 131]. NETs were found to be induced by opportunistic fungi such as *C. albicans* [130] in a human in vitro study that demonstrated that NETs interact with yeast in both the single-cell form as well as the multicellular hyphal form, and incapacitate both forms via the action of the granular components of the NETs [130].

In contrast to the protective immune response exemplified by Th1 and Th17 cells [132], Th2 effector cells are considered deleterious in lung fungal infections, in part because they dampen the protective Th1-cell responses. The detrimental Th2

responses in pulmonary fungal infections cause significant clinical problems because Th2 responses are closely related to allergic inflammation and asthma. One example of a detrimental fungal Th2-cell response in the lung is that generated by allergic bronchopulmonary aspergillosis, which can result from inhalation of the fungal spores of *Aspergillus* spp. [133]. Indeed, the severity of asthma is associated with the presence of *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* species in the lung, exposure to which may occur indoors, outdoors, or both [118]. In order to improve upon current treatments for invasive fungal infections, it is imperative to understand the nature of fungal pathogenesis not only in the context of the diversity of fungal strains present in the lung [134] but also the complex interplay between lung-colonizing microbial communities and invading pathogens.

As mentioned before, one notable component of the lung mycobiota of a healthy individual is *Pneumocystis* spp. [135]. New molecular surveys are revealing that *Pneumocystis* is carried at low levels, even in healthy individuals. This fungus can be spread from individual to individual through airborne transmission, but it can also cause pneumonia following overgrowth in HIV-immunocompromised hosts [136]. *Pneumocystis* has also been implicated as a cofactor of chronic obstructive pulmonary disease [137]. Thus, *Pneumocystis* appears to exist as a very low level commensal in the lung microbiota when the host is healthy and becomes pathogenic when the host becomes immunocompromised.

Cystic fibrosis (CF) provides an important example of the need to enhance our knowledge of the composition of the microbial community in order to improve management of patients susceptible to pulmonary infections. Using pyrosequencing, Delhaes et al. [138] extensively explored the diversity and dynamics of fungal and prokaryotic populations in the lower airways of CF patients. The authors identified 30 genera, including 24 micromycetes, such as *Pneumocystis jirovecii* or *Malassezia* sp., and six basidiomycetous fungi [138]. Among the organisms identified, filamentous fungi belonging to the genera *Aspergillus* and *Penicillium* had previously been suggested as pathogens in CF patients [139]. *Candida albicans* and *C. parapsilosis* were also recently described as colonizer organisms of CF patients [140, 141]. A significant proportion of other identified species were fungi also detected in patients with asthma (*Didymella exitialis*, *Penicillium camemberti*), allergic responses (*A. penicilloides* and *Eurotium halophilicum*) [142, 143], or infectious diseases (*Kluyveromyces lactis*, *Malassezia* sp., *Cryptococci* non-neoformans, *Chalara* sp.) [144]. Fungal colonization (especially repeated or chronic colonization) may thus have a substantial impact on the development of CF and other pulmonary diseases, but more studies are required to determine the real risk relative to the fungal component of the lung microbiota, especially because the coexistence of the bacterial component must be taken into account. A modification in lung mycobiota may result from a primary dysbiosis of bacteria transiently to persistently colonizing the lung [145, 146]. Indeed, the causative or the correlative relation between changes in lung mycobiota and disease onset needs to be proven by expanding the number of samples and moving forward the study from the species to the strain level.

Fungi inhabiting the GI mucosal surface: The gut mycobiota

The human GI tract is known to contain a variable fungal microbiota, but the phylogenetic characteristics of those fungal microorganisms and their specific roles as part of the GI tract ecosystem have not yet been studied extensively. Despite its harsh environment, the stomach harbors a microbiota that can include *Lactobacillus*, *Helicobacter*, and *Candida* spp. [147]. *Candida* colonization of the GI tract of mice has been shown to drive allergic sensitization to food Ags by affecting the mucosal barrier [148]. In particular, intragastrically inoculated mice were administered with OVA to assess Ag sensitization and GI permeability, and anti-OVA Ab titers and plasma concentrations of OVA were measured weekly. The authors showed that *C. albicans* promoted allergic sensitization was due to mast cell mediated hyperpermeability in the GI mucosa [148]. In healthy human volunteers, another group carried out both culture-independent analyses, based on DNA extraction and PCR targeting of both total eukaryotic 18S rRNA genes and fungal ITS, together with culture-dependent analyses of fungi [19]. This study found that the eukaryotic diversity of the human gut is low, largely temporally stable, and dominated by various subtypes of *Blastocystis* and *Candida* [19]. The low diversity is likely an artifact due to the fact that the most abundant species occur in the cultivable fraction, particularly *Candida* spp. The culture-independent analysis revealed a greater number of genera, such as *Gloeotinia/Paecilomyces* and *Galactomyces*, suggesting the importance of using culture-independent surveys to assess species composition [19]. An example of the large variability of the human gut mycobiota was recently provided by a study of four children and their respective mothers, which reported that infants harbor *Saccharomyces* spp. as opposed to *Candida* as the most frequent fungal species in the gut (36%) with respect to their mothers [149].

Whether *S. cerevisiae* is present in the human gut at birth remains to be elucidated. It is possible that yeasts simply reach the GI tract through food. Fermented foods and beverages containing eukaryotic species such as bread, beer, and wine are consumed on a daily basis, providing ready inocula for the host [19]. Alternatively, it is possible that differences in fungal colonization are related to differences in the genetic makeup of the host or differences in gut permeability.

The numerous and diverse interactions between fungi, bacteria, and immune responses can significantly impact gut health and likely contribute to the pathobiology of GI disorders from irritable bowel syndrome to IBD. When mucosal homeostasis breaks down in a genetically predisposed individual, the resulting immune response may lead to chronic inflammation [150]. Antibodies against *S. cerevisiae* have been shown to be disease marker for Crohn's disease (CD) [151], possibly indicating that fungi could play a role in the aberrant immune responses in IBD [152]. A few studies have been conducted to examine fungal community dysbiosis in chronic disease, including that in IBD [16, 153]. Fungal diversity in the large intestine of patients with CD is higher than that seen in healthy subjects [16]. The study of the mycobiome

in a murine model of induced colitis highlighted the importance of the gut mycobiota in contributing to the boost in intestinal inflammation seen upon dextran sodium sulfate (DSS) treatment [152], with a marked increase in the abundance of *C. tropicalis* observed during active colitis. These studies are the first steps toward clarifying the role of the gut mycobiota in intestinal inflammation, and may help explain the increased serum levels of anti-*S. cerevisiae* antibodies in CD patients [151]. A number of other opportunistic infections are generally ascribed to defective host immunity but may require specific microbial population dysbiosis [153]. Longitudinal molecular typing studies indicate that disseminated *C. albicans* infections originate from an individual's own commensal strains [154], and the transition to virulence is generally thought to reflect impaired host immunity. However, recent data indicate that the ability of a commensal organism to produce disease is not merely a consequence of impaired host immunity. Suzanne Noble and colleagues [155] showed that the opportunistic pathogen *C. albicans* can enter a specific, regulated commensal state called GUT (gastrointestinally induced transition) in the host intestine. *Candida albicans* in the GUT state have a unique phenotype that promotes carriage in the gut in a benign state, in which virulence-associated genes, such as the white-opaque switching and hyphal formation genes, are downregulated, enabling fungal adaptation for long-term survival in the large intestine [155]. Nevertheless, GUT cells can promote pathogenesis when host immunity is impaired. These new findings suggest that more attention will be directed toward understanding fungal persistence, colonization, and commensalisms — processes that may have evolved over many thousands of years of coevolution within the human host.

Diet is a constant and dynamic factor shaping mucosal immunity as well as the composition of resident microbial populations in the gut. To maintain gut homeostasis, immune cells must sample Ags from the intestinal lumen and deliver them to lymph nodes for presentation to T cells (Fig. 1). In the lymph nodes, CX3CR1⁺ macrophages and CD103⁺ DCs collaborate in a fascinating way to capture soluble food Ags [156] and induce oral tolerance. In addition, ILC3s control the production of cytokines important for control of fungal burden, such as IL-17 and IL-22 [157]. Hoffmann et al. investigated the association of diet with fungal populations, using fecal samples from 98 healthy individuals [158]. They characterized 62 fungal genera and 184 species by deep sequencing, and usually found that the presence of either the phyla Ascomycota or Basidiomycota was mutually exclusive. The authors could not conclude which of those fungi are true gut residents and which are passengers resulting from diet. We cannot exclude the possibility that the presence of *Saccharomyces* is due to the ingestion of yeast-containing foods such as bread and beer [82]. A recent study conducted on the Wayampi Amerindian community showed a high diversity among yeast species in the gut, with a prevalence of *S. cerevisiae* over *Candida* species [80], suggesting a role for this fungus in gut immune homeostasis. Thus, integrating information on the repertoire of the gut mycobiota in the context of the broader microbiota and developing functional tests to measure

its role in shaping immune function is necessary to better understand the role of the microbial communities in sustaining human health.

Microorganisms and the immune system exhibit crosstalk between the gut and various organs

Although we have described the composition of the fungal microbiota in various locations in the human body, we remain aware that these locations are not isolated and that DCs trained in the Peyer's patches of the intestine (Fig. 1) can shape T-cell responses in other locations. A clear example of this crosstalk was recently shown in an elegant study by Kim et al. [159], who showed that antibiotic treatment of mice increases susceptibility to allergic airway disease by promoting varying degrees of fungal outgrowth in the intestine, ultimately resulting in the acquisition of an M2 phenotype by alveolar macrophages [159]. The authors isolated *C. parapsilosis* from the feces of antibiotic-treated mice and showed that transferring this fungus to mice that did not carry this species increased their susceptibility to allergic airway inflammation induced by papain or house dust mite extract [159]. Oral treatment of mice with *Candida* species isolated from humans also led to fungal outgrowth in the gut and exacerbated allergic airway inflammation, increasing serum levels of prostaglandin E₂, which promoted the development of M2 macrophages [159]. The mycobiota alteration mediated imbalance in alveolar macrophage function contributed to the increase in airway inflammation, as untreated animals receiving alveolar macrophages from antibiotic-treated mice developed more severe airway inflammation than animals that received alveolar macrophages from control mice. Based on this result, it appears that intestinal dysbiosis, particularly the altered ratio of fungi to bacteria, could be a causative factor in the development of allergic disease. Patients with severe asthma with fungal sensitization are often sensitized to *C. albicans* and benefit from antifungal drug therapy [160]. Colonization of mice with *C. albicans* has been shown to result in dietary Ag leak from the stomach [148]. CD is associated with a microbiotic dysbiosis and the development of antibodies against members of the microbiota [161]. This includes anti-*S. cerevisiae* antibodies, which have been shown to be reactive to an in vivo expressed epitope on *Candida* species, as well as baker's yeast [149]. Defects in the C-type lectin, β -glucan receptor dectin-1 — which plays a fundamental role in antifungal immunity by β -glucan yeast wall component recognition [162] and which deficiency in humans causes fungal infection susceptibility [50] — confer increased susceptibility to chemically induced colitis, disease that could be exacerbated by repeated oral delivery of *C. tropicalis* [160]. This was consistent with the report that *C. albicans* could also exacerbate DSS-induced colitis [163] and that an indigenous *Candida* population could drive disease. Similarly, lung responses generated via the β -glucan receptor dectin-1 are required for lung defense during acute, invasive *A. fumigatus* infection through IL-22 production [164]. Unexpectedly, lung responses generated via dectin-1, in an

allergic mouse model of chronic lung exposure to live *A. fumigatus* conidia, lead to the induction of multiple proallergic (Muc5ac, Clca3, CCL17, CCL22, and IL-33) and proinflammatory (IL-1 β and CXCL1) mediators that compromised lung function [165]. Assessment of cytokine responses demonstrated that purified lung CD4⁺ T cells produced IL-4, IL-13, IFN- γ , and IL-17A, but not IL-22, in a dectin-1-dependent manner [108]. Overall we can conclude that dectin-1 contributes to both protection and gut and lung inflammation and immunopathology associated with persistent fungal exposure, via mechanisms that involve constant integration of messages derived from different locations in the body.

Conclusions

Recent culture-independent surveys of eukaryotic communities reveal that, similar to bacteria, commensal fungi are an essential part of human ecosystems. The role of the mycobiota in the maintenance of health can be understood only using a “systems level” integrated ecological approach, as opposed to an approach focused on specific, disease-causing taxa. Strain-specific traits, such as differences in cell wall composition among isolates from the same fungal species, may prove to be as important as differences in mycobiota species composition to maintain the correct immune homeostasis [134, 166]. Previous results demonstrating a switch from a Th1-Treg response to a Th17 response following exposure to different life stages of the same strain of *S. cerevisiae* [167], as well as the results showing the *Candida* GUT phenotype shift [155] are clear examples of the need to functionally analyze the mycobiota at the strain level, rather than simply counting its parts at the species level.

This analysis will require sequencing and annotating many more fungal genomes than are currently known, developing strains specific markers as well as gaining an understanding of their population and community structures in the context of the evolutionary forces shaping their adaptation to the host. High-throughput analysis of fungal cells walls as well as in-depth sequencing of the meta-transcriptome of eukaryotes during the interaction with the host immune system will soon offer a novel window into the integrated functioning of the mycobiota and microbiota.

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Abbreviations: AD: atopic dermatitis · AhR: aryl hydrocarbon receptor · CD: Crohn's disease · CF: cystic fibrosis · GI: gastrointestinal · GUT: gastrointestinally induced transition · IBD: inflammatory bowel disease · ILC3: innate lymphoid type 3 cell · ITS: internal transcribed spacer · LC: Langerhans cell · NET: neutrophil extracellular trap

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