Human Gut Mycobiome in IBD

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The human microbiota is a diverse microbial ecosystem associated with many beneficial physiological functions, as well as numerous disease etiologies. Dominated by bacteria, the microbiota also includes commensal populations of fungi, viruses, archaea, and protists. Unlike bacterial microbiota, which was extensively studied in the past two decades, these non-bacterial microorganisms, their functional roles, and their interaction with one another or with host immune system have not been as widely explored. This review covers the recent findings on the fungal communities of the human gastrointestinal microbiota, termed the “mycobiome”, and their involvement in health and disease, with particular focus on the pathophysiology of inflammatory bowel disease.

gut mycobiome | inflammatory bowel disease (IBD)

Introduction

Fungi are ubiquitous in the environment and a part of all Earth’s ecosystems[1]. In addition, a diverse population of commensal fungi has been recognized as a fundamental component of the human body, co-existing with other microbes within the human microbiota.[2] In contrast to the vast number of studies on the bacterial communities of the microbiota conducted in the last decades, the fungal constituents of the microbiota, the mycobiome, received much less attention. Still, recent research acknowledged human mycobiome as a dynamic community, responsive to environmental and pathophysiological changes, and playing a vital role in host metabolism as well as in maintenance of host immune homeostasis.[2][3][4][5] Human mycobiome is also implicated in various disease conditions, including inflammatory bowel disease (IBD) and its two main entities: Crohn’s disease (CD) and ulcerative colitis (UC). [6][7][8][9]

Human mycobiome research

Early research of human mycobiome was based on culture-dependent techniques for the identification and characterization of commensal fungal communities. While the new molecular culture-independent next-generation sequencing (NGS) techniques proved very effective for analyzing the bacterial component of microbiota, the DNA-based sequencing studies of the human mycobiome are faced with several limitations. Fungi account for a relatively small percentage of the human microbiota, with 10^5 to 10^6 fungal cells per gram of fecal matter (compared to 10^{11} bacterial cells per gram)[10] and only 0.1% of the 9.9 million reference genes in a current human gut microbial metagenomic reference catalog are reported to be of eukaryotic origin.[11][12] Additionally, the identification of composition and diversity of the fungal community is influenced by the nucleic acid isolation

method,[13] the choice of sequencing primer pairs,[14] as well as different sequencing technologies[15][16] and bioinformatics pipelines.[17][18] Finally, the incomplete databases for taxonomic assignment and annotation of fungal genomes present a serious difficulty in studying the human mycobiome.[14]

The usual molecular target for identifying fungi are the internal transcribed spacer (ITS) regions of ribosomal RNA genes. As the ITS regions are highly divergent among fungi, these regions are often sufficiently different to classify fungi to the species level. In 2012, ITS was designated as the universal DNA barcode marker for the kingdom Fungi,[19] although this approach revealed potential PCR biases.[20][21] A recent study proposed adding translational elongating factor 1α (TEF1α) as a secondary barcode to the ITS barcode in order to increase the taxonomic resolution power and enhance the accuracy of fungal species identification.[22] On the other hand, the study comparing 18S rRNA screening to ITS sequencing showed higher sensitivity of 18S rRNA RT-PCR combined with SANGER sequencing, as this method detected fungal communities in several samples which were ITS negative.[23] Currently, there is no consensus on the best methodological approach for identifying human mycobiome, and consequently the results of studies using different methods vary.

Human gut mycobiome

Human mycobiome inhabits the gastrointestinal tract but also skin, respiratory tract, genitourinary tract, as well as other mucosal surfaces in the host. The gastrointestinal tract is the most studied fungal niche in humans. Reports suggest that the human gut is populated by three fungal phyla, *Ascomycota*, *Basidiomycota*, and *Zygomycota,*[24][25] with the “core” 10 genera identified in the majority of gastrointestinal tract samples consisting of *Candida* (particularly *C. albicans*), *Saccharomyces* (particularly *S. cerevisiae*), *Penicillium*, *Aspergillus*, *Cryptococcus*, *Malassezia* (particularly *M. restricta*), *Cladosporium*, *Galactomyces*, *Debaryomyces*, and *Trichosporon.*[24][26] The composition of gut mycobiome seems to be dynamic over time and far more variable than the composition of bacteria, both in humans[27] and in mice.[28] Most studies consider fungi as commensal organisms in the gut, acquired early in life.[29] This has recently been challenged claiming that fungi do not routinely colonize the gastrointestinal tract of healthy adults,[30] instead postulating that all fungi identified in the human stool samples could be explained by their presence in the mouth or the diet. Indeed, diet is perceived as a crucial factor affecting the composition and variability of gut mycobiome.[31] For instance, gut mycobiome content was found to considerably differ between individuals having different dietary patterns, i.e., vegetarians and people on a conventional Western diet.[24][31] Additionally, reports suggest that the abundance of *Candida* in the gut positively correlated with high carbohydrate diets, and inversely correlated to consumption of total saturated fatty acids, while recent intake of short-chain fatty acids reduced the abundance of *Aspergillus.*[25] Another notable finding of this study was the co-occurrence of *Candida* with particular bacterial (*Prevotella* and *Rumminococcus*) and archaeal genera (*Methanobrevibacter*), providing support for the interkingdom syntrophic relationships in host metabolism.

One of the first indications that fungi play a role in modulating gut homeostasis is the use of *Saccharomyces boulardii* as a constituent of herbal medicine traditionally utilized in Southeast Asia to reduce the severe diarrhea in patients with cholera. *S. boulardii* is still prescribed as a probiotic to prevent diarrhea and intestinal colonization with *Clostridioides difficile* following antibiotic therapy[32][33] and is efficient in preventing recurrent *C. difficile*

infections.[34] The positive effects of *S. boulardii* come from inactivating pathogen toxins and directly inhibiting the growth and invasion of intestinal pathogens,[35][36] as well as boosting the host immunity and exerting anti-inflammatory functions in ulcerative colitis,[37][38] Crohn's disease,[37][39] and *C. difficile* colitis.[40] A recent report suggests beneficial effects of another probiotic yeast, *Candida kefyr*, in reducing the severity of colitis in animal models by decreasing the abundance of *Bacteroides* and lowering IL-6 production, thus attenuating inflammation in the intestine.[41]

Although fungi can exert beneficial effects to host health, the disturbance of gut mycobiota was also implicated in various gastrointestinal diseases. A recent study demonstrated no significant changes in mycobiome richness between obese and non-obese subjects; however, some specific compositional differences were noted. The most prevalent genus in non-obese individuals was *Mucor*, with its abundance significantly higher in non-obese individuals, and inversely correlated with metabolic markers of obesity.[42] In colorectal cancer (CRC), an alteration of fungal composition and ecology was observed, characterized by an increased *Basidiomycota*/*Ascomycota* ratio, depletion of *S. cerevisiae*, as well as enrichment of *Rhodotorula*, *Malassezia*, and *Acremonium* genera along with several *Aspergillus* species (including *A. flavus*, a major producer of highly toxic carcinogen aflatoxin), suggesting their possible contribution towards CRC pathogenesis.[43] Insights into gut mycobiota playing a role in irritable bowel syndrome (IBS) were also reported. Decreased fungal diversity and dysbiosis were found in IBS patients, correlating mycobiota signature with visceral hypersensitivity, which is considered as one of the major pathophysiological features of IBS.[44] Interestingly, treatment with fungicides could recover the visceral hypersensitivity to normal levels.[43] This finding is in accordance with a previous study that reported yeast-free diets and antifungal treatments as helpful for IBS subjects. [45] In addition, *S. boulardii* was found to be effective in improving symptoms and the quality of life in IBS patients.[46]

Human gut mycobiome in IBD

The majority of research on the effects of gut mycobiota in gastrointestinal diseases was however concentrated on intestinal inflammation and IBD (Table 1). Even before the advent of molecular methods and next-generation sequencing (NGS), increased levels of anti-*S. cerevisiae* antibodies (ASCA) were commonly found in the serum of CD patients, suggesting the host's immune responses toward intestinal fungi.[47] These antibodies, raised against mannan, a component in the fungal cell wall, were soon identified as a reliable diagnostic biomarker for CD and predictors of the disease course.[48][49] ASCA also recognize many other fungi, including *Candida.*[50] Indeed, reduced fungal diversity and significantly increased abundance of specific *Candida* species were found in pediatric IBD patients.[51] Sokol et al. report a similar finding in adult subjects with IBD: a decrease in gut mycobiome biodiversity and elevated *Basidiomycota*/*Ascomycota* ratio, mainly due to the increased prevalence and abundance of *C. albicans* and reduction of *S. cerevisiae.*[6] Additional studies confirmed an increased representation of *Candida* species in IBD, namely *C. tropicalis* in familial CD,[7] as well as *C. glabrata* in colonic biopsy samples from patients with CD.[8] Besides elevated *Basidiomycota*/*Ascomycota* ratio in IBD patients in comparison to healthy controls and in IBD flares vs. IBD remission,[6] fungal dysbiosis in IBD patients is also characterized by increased levels of *Gibberella moniliformis*, *Alternaria brassicola*, *Aspergillus clavatus*, and *Cystofilobasidiaceae,*[8] while

Saccharomyces cerevisiae and *Malassezia sympodialis* are markedly decreased.[6] Additionally, studies confirm fungal burden is increased in both CD and UC,[8][9] with the fungal cells translocating trough the intestinal barrier during the chronic stage of colitis.[9][52]

Table 1. Major contributors of mycobiome changes in IBD.

IBD type	change	reference
CD + UC	↑ *Basidiomycota*/*Ascomycota* ratio	[6]
CD + UC	↑ *Candida albicans*	[6]
CD	↑ *Candida tropicalis*	[7]
CD	↑ *Candida glabrata*	[8]
CD	↑ *Gibberella moniliformis*	[8]
CD	↑ *Alternaria brassicola*	[8]
CD	↑ *Aspregillus clavatus*	[8]
CD	↑ *Cystofilobasidiaceae* family	[8]
CD + UC	↓ *Saccharomyces cerevisiae*	[6]
CD + UC	↓ *Malassezia sympodialis*	[6]
UC	↓ fungal diversity	[6]
CD + UC	↑ fungal burden	[8][9]

UC	↑ fungal-bacteria interactions	[6]
CD	↓ fungal-bacteria interactions	[6]

Some of the studies simultaneously analyzed both the fungal and bacterial microbiota revealing that the intestinal microbial network was different in IBD patients when compared to healthy individuals. Sokol et al. identified positive correlations between the decreased abundance of *S. cerevisiae* and reduction of several bacterial genera, such as *Bifidobacterium*, *Blautia*, *Roseburia*, and *Ruminococcus*. The total number and the intensity of fungal–bacterial associations were increased in UC, with distinct interactions potentially involved in the inflammatory processes. On the other hand, weaker fungal–bacteria correlations were found in CD when compared to healthy volunteers, implying disrupted connections between two kingdoms in this disease.[6] A study by Hoarau et al. reported elevated levels of *C. tropicalis* positively correlated with *Serratia marcescens* and *Escherichia coli* in CD. Moreover, in vitro experiments confirmed these species form thicker mixed biofilm than any of the species generates individually, creating a commensal niche additionally enriched in fungal hyphae, a form of growth usually implicated in pathogenic conditions.[7] The fact that interactions between gut bacteria and fungi are closely associated with disease was also investigated in mouse models of dextran sulfate sodium (DSS) induced colitis. Qiu et al. found that inflamed mouse intestine contained increased fungal burden in the mucosa, but decreased in the feces. The dysbiosis was characterized by elevated *Wickerhamomyces*, *Alternaria*, and *Candida*, together with reduced *Cryptococcus*, *Phialemonium*, and *Wallemia*, and unidentified *Saccharomycetales* genus.[51] The study further shows mice with fungi depleted by fluconazole treatment exhibited aggravated colitis, in contrast to bacteria-depleted mice, that showed alleviated intestinal inflammation and a trend of disease remission. This finding suggests that bacteria are the major driving force in acute inflammation and fungi may act as a counterbalance in maintaining the microbial homeostasis in acute colitis. In chronic recurrent colitis however, fungi may aggravate the disease severity and translocate into locations outside the gut.[51] A recent study by Sovran et al. identified opposing effects of administrating *C. albicans* or *S. boulardii* to mice with DSS-induced colitis, resulting in increased disease severity or reduced disease symptoms, respectively. However, broad-spectrum antibiotic treatment protected the mice from colitis and *C. albicans* had no pro-inflammatory effect when administered to mice with disrupted bacterial microbiota, suggesting bacteria are essential for the development of colitis and *C. albicans* requires the presence of specific bacteria that trigger the intestinal inflammation to increase the disease intensity. On the other hand, mice with depleted *Enterobacteriaceae* exhibited normal susceptibility to colitis, but neither *C. albicans* nor *S. boulardii* could exert disease-modulating effects in this experimental setting. After reintroducing *Enterobacteriaceae*, both *C. albicans* and *S. boulardii* recovered their effects in severity of colitis.[53]

The host immune system recognizes fungi using pattern recognition receptors (PRRs), with the resulting host responses ranging from tolerance to inflammation. The key PRR for coordinating host response to fungi is Dectin-1 (CLEC7A), a C-type lectin receptor that recognizes β-glucans in the fungal cell wall.[54] Dectin-1 activates macrophages and dendritic cells, initiates phagocytosis of fungi, and induces signaling cascade via caspase-associated recruitment domain-containing protein 9 (CARD9) and NF-kB to produce pro-inflammatory cytokines. A

recent study demonstrated a central role of Dectin-1 in regulating the severity of inflammation in mouse models of DSS-induced colitis.[55] Dectin-1 deficient mice were found to develop more severe colitis, due to the overgrowth of opportunistic fungi (i.e., *Candida* and *Trichosporon*), while treatment with antifungal drug fluconazole ameliorated the disease.[54][56] The same study revealed that a polymorphism in the Dectin-1 gene was associated with increased severity of disease in patients with UC.[54] Recent research also identified CARD9 as the key downstream signaling molecule for the induction of immune response to fungi.[57] CARD9-deficient patients are especially susceptible to fungal infections and polymorphism in CARD9 gene is associated with a higher risk of developing IBD.[58] Interestingly, *Candida* overgrowth, which is one of the characteristic features in IBD patients, could not be positively correlated with CARD9 polymorphism.[59] Instead, *Candida* was hardly detectible in CARD9 deficient mice, suggesting this taxon was not the driver of dysbiosis as in dectin-1 deficient animals.[60] IL-17 and IL-22 were also found to affect commensal fungal communities. A clinical study revealed secukinumab, an IL-17A antagonist, was associated with exacerbations in patients with CD, identifying the higher rate of fungal infections in treated subjects.[61] Both IL-17 and IL-22 might act as inducers of antimicrobial peptides (AMPs) in epithelial cells and were reported as protective against mucosal fungal infections.[62][63]

Conclusion

The significant role of mycobiome in maintaining human homeostasis, as well as in disease etiology, is slowly unveiling. The impact of the diverse fungal communities on human health needs to be determined in more detail in order to expand the current “bacteriocentric” view of human microbiota and provide more holistic understanding of the human superorganism. To achieve this task, two important prerequisites are essential: (1) expanding fungal reference genomes in the currently available databases for reliable identification of those microorganisms; (2) establishing uniform methods of detection for fungal commensal populations to ensure consistent and comparable evaluation of fungal abundance in different human body sites. The improved tools and the newly generated data would provide deeper insight into human mycobiome and the possibilities of its exploitation in promoting human health and ameliorating disease. Although microbiome-directed therapy is still in its infancy, studies conducted thus far suggest that direct or indirect alterations in human mycobiome may improve health outcomes in inflammatory diseases such as IBD.

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