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REVIEW



The microbiome-metabolome crosstalk in the pathogenesis of respiratory fungal diseases

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ABSTRACT

Filamentous fungi of the genus *Aspergillus* are responsible for several superficial and invasive infections and allergic syndromes. The risk of infection and its clinical outcome vary significantly even among patients with similar predisposing clinical factors and pathogen exposure. There is increasing evidence that the individual microbiome supervises the outcome of the host-fungus interaction by influencing mechanisms of immune regulation, inflammation, metabolism, and other physiological processes. Microbiome-mediated mechanisms of resistance allow therefore the control of fungal colonization, preventing the onset of overt disease, particularly in patients with underlying immune dysfunction. Here, we review this emerging area of research and discuss the contribution of the microbiota (and its dysbiosis), including its immunoregulatory properties and relationship with the metabolic activity of commensals, to respiratory fungal diseases. Finally, we highlight possible strategies aimed at decoding the microbiome-metabolome dialog and at its exploitation toward personalized medical interventions in patients at high risk of infection.

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Introduction

Aspergillosis comprises a wide spectrum of fungal diseases caused by *Aspergillus* spp. with clinical manifestations that range from colonization, to allergic syndromes, to invasive forms of infection.¹ The increased success of solid organ and stem-cell transplantation and cancer chemotherapy has, paradoxically, resulted in a rapidly expanding population of immunocompromised patients, which display a distinctive susceptibility to invasive aspergillosis (IA).² Patients suffering from chronic obstructive pulmonary disease (COPD) or influenza infection under intensive care are also at risk of developing IA.^{3,4} Besides invasive disease, patients with asthma or cystic fibrosis (CF) are prone to develop fungal-induced allergic airway diseases, the most severe form being allergic bronchopulmonary aspergillosis (ABPA).⁵ In addition, chronic pulmonary aspergillosis (CPA) is a typical feature of patients with pre-existing cavities caused by tuberculosis or COPD.⁶

The excessive prescription of antifungal drugs and the emergence of resistant strains, as well as the remarkable burden conveyed by these diseases to the healthcare

systems have driven efforts at an improved understanding of their pathogenesis. An emerging body of evidence has highlighted the significant role of the pulmonary microbiome in inflammation, metabolism, and other physiological processes that regulate the antifungal immune response and condition host susceptibility to diseases caused by *Aspergillus* spp. (Fig. 1).

The microbiota is defined as the ecological community of commensal, symbiotic and pathogenic organisms that inhabit the lungs,^{7,8} and it includes 6 predominant phyla: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria and Cyanobacteria.⁸ Despite such diversity, the microbial density in the lungs is estimated at around 10⁴ bacterial cells/mL,⁹ a considerably lower value than that reported for the oral cavity (10¹¹/mL),¹⁰ skin (10⁷/cm²)¹¹ or gut (10¹²/mL)¹² microbiomes. Until recently, the lung was thought to be a sterile organ mainly because of limitations of standard microbial culture techniques to mimic the lung habitat. The development of cutting-edge molecular methods for the quantification and sequencing of bacterial DNA has revealed however that the airways harbour a unique

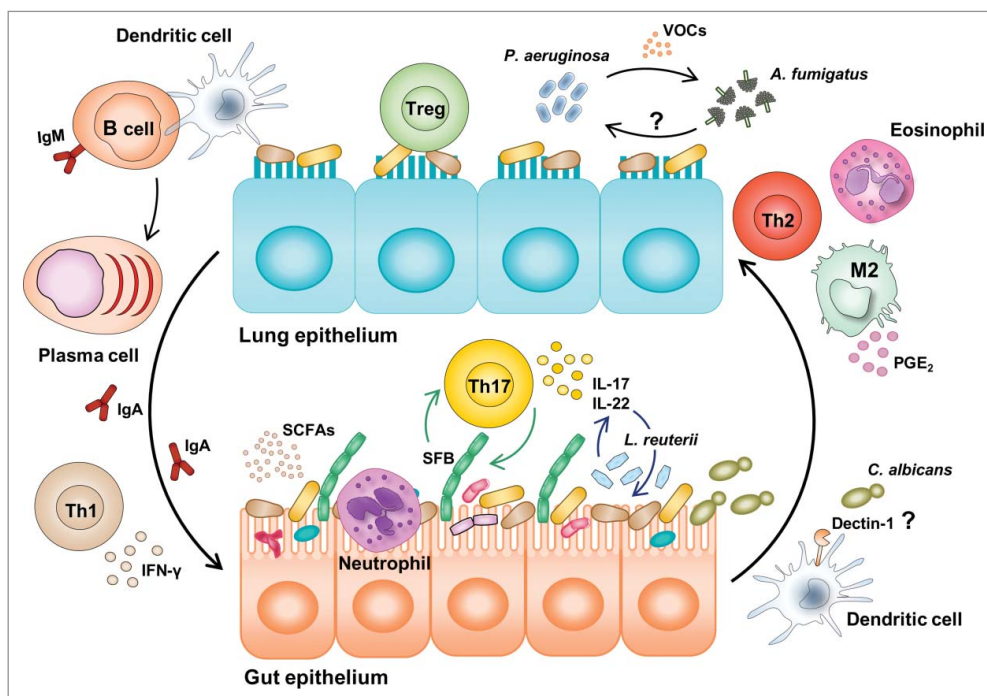


Figure 1. The contribution of the microbiome-metabolome crosstalk to host immunity against *A. fumigatus*. The function of the immune system is regulated by the microbiota and its metabolic activity, whereas the diversity of the microbiota and its commensal nature are kept under control by the immune system. The gut dysbiosis is often associated with the outgrowth of the yeast *C. albicans* that, in turn, leads to the development of allergic airway responses to *A. fumigatus* mediated by Th2 cells, M2-polarized macrophages capable of producing inflammatory mediators such as prostaglandin E2 (PGE₂), and eosinophils. Segmented filamentous bacteria (SFB) are known to regulate Th17 immunity locally in the gut but also in the lung in response to *A. fumigatus*, and gut microbiota-derived metabolites such as short-chain fatty acids (SCFAs) have been identified as master regulators of pulmonary immune responses. In addition to these, lactobacilli are able to degrade tryptophan into immunoregulatory metabolites and induce the production of IL-22 to sustain immune tolerance to *C. albicans*. The microbial communities inhabiting the lung are, in turn, able to regulate gut immunity, for example by promoting the expansion of IFN- γ -producing Th1 cells and recruitment to the gut, and inducing IgA class switch recombination and systemic humoral responses. Some of the bacterial species in the lung, most notably *P. aeruginosa*, interact directly (and indirectly via metabolites, including volatile organic compounds, VOCs) with *A. fumigatus*, contributing to the establishment of a permissive environment for fungal colonization, and under certain conditions, to overt disease.

dynamic microbiota,¹³ with Bacteroidetes and Firmicutes being the most predominant phyla,^{14,15} highlighting the need to accommodate an additional layer of complexity in the pathogenesis of respiratory diseases.

Despite recent research in this field, the existence of a resident lung microbiota has still to be clearly demonstrated.^{9,16-18} The healthy lung occasionally contains bacterial species traditionally associated with the oral cavity,^{7,9,17,19} suggesting that the local microbiota likely reflects the migration of bacterial communities from neighboring niches.²⁰ Differences in commensal and pathogenic microbial loads in the airways are therefore possibly influenced by the rate at which new bacterial species enter the lung and the rate at which these are able to colonize or go extinct. Although healthy and diseased lungs are equally reachable by microbes from the surrounding environment, the mucociliary clearance often impaired in many chronic inflammatory airway diseases²¹ may represent a major permissive factor for

adaption and establishment of species colonizing the lower airways.²⁰ The persistence of specific oral taxa in diseased lungs may also occur due to the establishment of a pro-inflammatory microenvironment.²²

The co-evolution of the microbiota with the innate immune system and its various microbe-sensing pattern recognition receptors (PRRs) has resulted in elaborate crosstalk mechanisms through which both systems control mutual homeostasis.^{23,24} While microbial recognition by PRRs is required for a stable microbial composition, the presence (and activity) of the microbiota is, in turn, necessary for the development and function of the immune system.^{25,26} When working properly, this immune system-microbiota coalition promotes protective responses to pathogens, establishes tolerance to innocuous antigens and controls the overgrowth of indigenous pathobionts.²⁷ This is further supported by studies in newborn mice that disclosed important developmental dynamics in the lung microbiota associated

with a specific population of T regulatory cells (Tregs) conferring tolerance to bacterial antigens derived from the populating communities.²⁸

Many genetic defects of PRRs have been associated with susceptibility to infectious diseases.²⁹ Likewise, several associations between genetic defects in innate immunity and risk of aspergillosis have been disclosed.^{30,31} Given that many microbial taxa are highly heritable and that a strong genetic component defines the human microbiota,³² an intricate interaction between host genetics, the microbiota and its metabolic products, and the mucosal immune system is proposed. In this review, we discuss the regulatory crosstalk of the gut and lung microbiota and its inherent metabolic activity in the context of respiratory fungal diseases.

The gut-lung microbiota axis and pathogenesis of fungal diseases

The homeostasis in the composition of the normal flora in the respiratory tract is essential to prevent the expansion of species with pathogenic potential.³³ When dysbiosis occurs due to underlying pulmonary diseases, immune system dysfunction, or defects in the ciliary activity of the mucous pulmonary epithelium, fungal colonization in the lungs may become uncontrolled and exacerbate into overt fungal disease. Thus, the underlying conditions typically associated with aspergillosis and their altered microbiome profiles could provide significant insights into microbiota-driven mechanisms of pathogenesis (Fig. 2).

The complex communities of microbiota that inhabit environments such as the lung, skin, or gut are now appreciated for their role in maintaining organ, tissue, and immune homeostasis through both compartmentalized and systemic control of immunity to pathogens. For example, protective immunity against cutaneous leishmaniasis was found to critically depend on the skin, but not the gut, microbiota and its ability to regulate T-cell function downstream of interleukin (IL)-1 receptor (IL-1R).³⁴ Activation of IL-1R by commensal microbiota-derived signals appears to be one major mechanism providing control of host immunity. Indeed, IL-1R antagonist (IL-1Ra)-deficient mice can be protected from spontaneous arthritis by housing under germ-free conditions,³⁵ suggesting that the microbiome is responsible for the inflammatory phenotype underlying arthritis and other autoimmune diseases.

In other situations, it is well established that dysbiosis of the gut microbiota can influence immune responses at distal sites, including the lung.³⁶ The disruption of the microbiota following antibiotic treatment and concomitant gut colonization by *Candida albicans* was found to

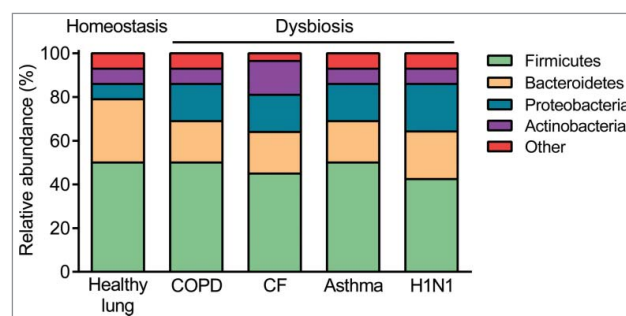


Figure 2. Bacterial dysbiosis in lung diseases associated with increased risk of infection and allergic reactions caused by *Aspergillus* spp. The microbial density in the lungs is estimated at around 10^4 bacterial cells/mL. The box plots summarize the relative bacterial abundance (%) typically detected in samples such as sputa, bronchoalveolar lavage or oropharyngeal fluids sampled from healthy and diseased lungs.^{13,33,109–111} Bacterial diversity was determined by 16S sequencing in most studies. Lung diseases such as COPD, influenza infection, CF and asthma influence the diversity and metabolic activity of the airway microbiota. The bacterial dysbiosis is associated with the outgrowth of certain bacterial communities at the expense of others (typically belonging to the Bacteroidetes phylum) and these altered profiles may be permissive for fungal colonization and disease. Patients with COPD and asthma share many resemblances in the bacteria causing dysbiosis, with an outgrowth of the Proteobacteria phylum (e.g., *Haemophilus* spp.) and a shift toward an increase in the proportion of streptococci and staphylococci within the Firmicutes phylum. Shifts in the bacterial communities in the CF lung are different, with an outgrowth of the Proteobacteria (which includes *P. aeruginosa*, *B. cepacia* and *H. influenzae*) and Actinobacteria phyla. An enrichment in the abundance of the Proteobacteria (particularly *Pseudomonas* spp.) and Firmicutes phyla is observed during H1N1 influenza infection.

drive potent CD4(+) T-cell-mediated allergic airway responses to *A. fumigatus*.³⁷ In contrast, the therapeutic administration of probiotics to primates has been shown to increase the frequency of immunoglobulin (Ig)A-expressing B-cells in the colon and lymph nodes, likely contributing to mucosal immunity.³⁸ More recently, the microbiota was also found to regulate the ability of lung dendritic cells (DCs) to induce IgA class-switch recombination and elicit protective gastrointestinal immune responses.³⁹ Taken together, these findings highlight important implications of the microbiota in regulating protective immunity conferred by vaccination.

Invasive aspergillosis

COPD has recently been acknowledged as an important risk factor for IA in critically ill patients.^{40,41} Because of the impairment in mucociliary clearance that may influence the balance between persistence and elimination of bacterial communities,⁴² understanding the contribution of the lung microbiome to the development of IA in this

setting is of great interest. Despite the lack of coherence between the many studies investigating the pulmonary microbiome in COPD, mostly due to sampling variability and heterogeneous therapeutic regimens,³³ recent studies agree that specific bacterial populations, such as *Haemophilus* spp., *Streptococcus* spp. and *Staphylococcus* spp., are enriched in the airways of patients suffering from COPD.⁴³⁻⁴⁵ In addition, the large FUNGI-COPD study reported that *Aspergillus* spp. were frequently isolated from the sputum of COPD patients during acute exacerbations and that the concomitant isolation of *Pseudomonas aeruginosa* contributed to fungal colonization.⁴⁶ Nevertheless, and with the existing data, the question remains as to what extent the pulmonary dysbiosis in COPD accommodates fungal colonization and ultimately contributes to the development of IA.

Patients infected with influenza, particularly the H1N1 strain, represent another group of non-neutropenic patients at risk of IA.⁴ Microbiome analyses revealed an enrichment in the abundance of the Firmicutes and Proteobacteria phyla (particularly *Pseudomonas* spp) in the lungs of patients infected with H1N1.⁴⁷ Thus, H1N1 appears to skew the lung microbiome toward a permissive profile likely favoring secondary invasive infections including IA. In this regard, it is noteworthy that the characteristics of the pulmonary microbiota are aligned with distinct innate cell gene expression profiles after lung transplantation.⁴⁸ Whereas a non-polarized activation of macrophages was associated with a balanced microbial community, inflammatory and remodelling profiles of these immune cells were instead linked with bacterial dysbiosis.

Allergic bronchopulmonary aspergillosis

Chronic airway colonization by fungi has been reported in CF patients.⁴⁹ In this setting, the main clinical manifestation of fungal disease is ABPA, characterized by a severe hypersensitivity reaction to *Aspergillus* spp..⁵⁰ Recent studies targeting the composition of the airway microbiota showed a complex microbial diversity, with *P. aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, and *Burkholderia cepacia* representing the most abundant bacterial species, and *Candida* and *Aspergillus*, the most common fungal genera.^{49,51-54} This unique environment, together with the pulmonary dysfunction in CF patients due to mucus formation, oxygen tension and inflammatory cell recruitment, might favor the formation of bacterial and fungal biofilms and influence pathogenicity.⁵⁵ Of interest, some gut symbionts are able to cooperate with other commensals through a dedicated cross-feeding enzyme system, allowing them both to survive in the gut. For example, *Bacteroides ovatus* is able to

digest polysaccharides at a cost to itself, while benefiting other species.⁵⁶

Another important microbial interaction that takes place in the CF lung is the one between *P. aeruginosa* and *A. fumigatus*. A number of studies have proposed an antagonistic relationship between these organisms triggered by direct contact and release of small molecules affecting quorum-sensing networks (e.g., pyocyanin) and influencing the ability of *A. fumigatus* to germinate and develop biofilms.^{57,58} In contrast, antibiotic treatment targeting *P. aeruginosa* decreases the detection of *Aspergillus* spp. in sputum samples.⁵⁹ It will be interesting to assess in the future whether the fungus is also able to manipulate the polymicrobial flora in CF via its own metabolites. Of note, both *A. fumigatus* and *P. aeruginosa* are bound by the soluble PRR pentraxin 3 (PTX3), which in turn favors their recognition and clearance by the innate immune system.⁶⁰ Importantly, genetic variants influencing PTX3 expression in the lung have been associated with *P. aeruginosa* colonization in CF patients⁶¹ and IA in stem-cell and lung transplant recipients.⁶²⁻⁶⁴ Taken together, these observations suggest that PTX3 acts as a master regulator of microbiota homeostasis in the lung and that, under specific circumstances (e.g., the CF lung microenvironment), commensals may be able to subvert PTX3 expression in order to control conflicting species.

Fungal-induced allergic airway disease

Few studies to date have demonstrated a link between the microbiome and fungal-induced allergic airway disease or asthma. One example regards the antibiotic-induced disruption of the gut microbiota and colonization with *C. albicans*, and the ensuing allergic airway response to *A. fumigatus*, but not invasive disease, associated with enhanced T helper (Th)2 cytokines and eosinophilia.³⁷ Whether an enhanced priming of innate immunity receptors (e.g., dectin-1) resulting from the fungal outgrowth in the gut is specifically associated with the distal repercussions observed remains unexplored. For example, the microbiota are a source of peptidoglycan that persistently stimulates the innate immune system via the nucleotide oligomerization domain (NOD)1 receptor and enhances the killing of *Streptococcus pneumoniae* and *S. aureus*, ultimately establishing a mechanism of systemic immunomodulation by the microbiota.⁶⁵

More recently, gut dysbiosis induced by antibiotic treatment was found to promote allergic airway inflammation by shifting macrophage polarization in the lung toward the alternatively activated M2 phenotype.⁶⁶ Of note, antibiotic treatment resulted in the overgrowth of a

commensal species of *Candida* in the gut triggering increased plasma concentrations of prostaglandin E₂ (PGE₂). Suppression of PGE₂ synthesis with cyclooxygenase inhibitors suppressed M2 macrophage polarization and decreased allergic airway inflammatory cell infiltration in antibiotic-treated mice. Taken together, these findings suggest that alterations in the composition and diversity of both the gut microbiome and mycobiome may condition immune responses in the lung.

Microbiota-mediated regulation of antifungal immunity

An optimal host defense against *Aspergillus* relies primarily on professional phagocytes, such as neutrophils, macrophages and monocytes, and specific T-cell populations, including Th1, Th17 and Tregs that control the extent and the nature of the immune response.¹ Evidence indicates that the impact of host-commensal microbe interactions reaches far beyond the local environment and influences peripheral innate and adaptive immune function^{67,68} (Fig. 1). Accordingly, the intestinal microbiota has an immunoregulatory function expanding from the gut and may play a significant role in pulmonary diseases such as the various forms of aspergillosis. In particular, segmented filamentous bacteria (SFB) were found to be critically required for the induction of Th17 cells to produce IL-17 and IL-22 in the gut,⁶⁹ particularly due to their ability to adhere to intestinal epithelial cells.⁷⁰ Importantly, the role of intestinal SFB in the generation of pulmonary Th17 cells during experimental *A. fumigatus* infection was recently confirmed, an effect that was associated with systemic IL-1R signaling.⁷¹ Of note, Th17 cells and IL-17 receptor signaling were also found to reciprocally regulate the SFB burden, allowing a precise control of dysbiosis, Th17 immunity and susceptibility to autoimmune inflammation.⁷² It remains to be elucidated whether the lung microbiota also contains microorganisms with similar Th17-polarizing capacity as the SFB in the gut that might influence antifungal immunity in the lung.

The increased susceptibility of patients with influenza to IA⁴ suggests that the influenza virus itself is able to modulate the homeostatic microbiome and predispose to fungal disease. The gut microbiota, particularly neomycin-sensitive bacteria, regulates the immune defense against respiratory tract influenza A virus.⁷³ The fact that, in these conditions, a protective interferon (IFN)- γ production was impaired in antibiotic-depleted mice may suggest that the same bacterial populations may influence protective Th1 immunity in response to *A. fumigatus* directly or indirectly via the control of viral loads, ultimately predisposing to IA. In this regard, it is

noteworthy that influenza infection also promotes alterations in the intestinal microbiota with a reduction in *Lactobacillus* spp. and *Lactococcus* spp., and an outgrowth of Enterobacteriaceae.⁷⁴ This dysbiosis is mediated by IFN- γ -producing T-cells derived from the lung and recruited to the small intestine, an effect that is accompanied by the expansion of pathogenic Th17 cells in the gut underlying intestinal injury. Thus, it is plausible that IL-17 responses arising in the gut may further impact lung disease. The gut microbiota is also a critical regulator of type I IFN production during pulmonary viral infection⁷⁵ and these are key cytokines involved in antifungal immunity,⁷⁶ suggesting that the gut microbiota has indeed the potential to influence antifungal immunity. Whether the viral infection contributes to fungal disease or is merely a side-effect of the microbiota-induced immunological dysfunction is still unknown.

The gut microbiota has the capacity to alter pulmonary Th2 responses and predispose to allergic syndromes.^{37,77} Alterations in the gut microbiome by antibiotic treatment and delivery of *C. albicans*-induced immunoregulatory signals has been found to mediate allergic airway responsiveness to *A. fumigatus* even in the absence of systemic antigen priming.³⁷ Under these circumstances, the frequency of eosinophils and mast cells is increased, together with IL-5, IL-13, IgE, and mucus-secreting cells. In this regard, IL-22 was also found to play an important role in experimental fungal-induced asthma.⁷⁸ Besides Th17 cells, natural killer cells and innate lymphoid cells (ILCs) are able to produce IL-22 and have been implicated in the pathogenesis of asthma.⁷⁹ Specifically, ILCs are emerging as one key immune population with the ability to orchestrate microbiome-mediated immune regulation.⁸⁰ Thus, it is not surprising that IL-22 also acts as a master regulator of mucosal immunity with important roles in controlling the diversity of microbial communities, including colonization by *C. albicans*.⁸¹ Of note, caspase recruitment domain-containing protein (CARD)9-deficient mice were found to display a profound gut dysbiosis associated with the inability of *Lactobacillus* spp. to metabolize tryptophan and induce expression of IL-22.⁸² Thus, these data confirm that host genes affect the composition and function of the gut microbiota through the production of microbial metabolites, and points to the existence of a profound dysbiosis in patients harbouring mutations in the CARD9 gene suffering from severe fungal infections.^{83,84} In addition, sensing of fungal components via CARD9-mediated pathways may also be proposed as one key mechanism affecting the metabolic activity of the microbiota.

The fungal microbiome (mycobiome) is thought to represent as little as 0.1% of the total microbiome.⁸⁵

Potentially pathogenic fungi such as *Aspergillus* spp. and *Candida* spp. are contained within the mycobiome and are believed to expand and potentially contribute to disease upon disturbances in the environment or when the host is immunocompromised. Mice lacking dectin-1, the innate immune receptor for β -glucans present in the fungal cell wall, showed an increased susceptibility to colitis as the result of an increased proportion of opportunistic fungi belonging to the *Candida* and *Trichosporon* genera and impaired immune responses to these commensal fungi.⁸⁶ The fact that dectin-1 also recognizes β -glucans from *A. fumigatus*⁸⁷ raises the interesting possibility that a disruption at this level may also impact the pulmonary microbiome and the local immune response to the fungus. Indeed, genetic variants affecting dectin-1 have already been associated with the risk of developing IA in haematological patients^{88,89} and recipients of stem-cell transplantation.⁹⁰ In the future, the investigation of the microbiome of dectin-1-deficient patients might provide further insights into the genetic regulation of microbiota composition and function.

The microbiome-metabolome crosstalk

Nutrients and molecules derived from the metabolic activity of the microbiota may also provide signals to the host and potentially modulate antifungal immunity. The short-chain fatty acids (SCFAs), products of the intestinal microbiota that are recognized by the G-coupled protein receptor GPR43 expressed on innate immune cells,⁹¹ illustrate the pivotal role of microbial metabolites in the regulation of host immune responses. Mice lacking GPR43 were shown to be unable to resolve intestinal inflammation during experimental colitis,⁹² thereby demonstrating a molecular link between a host immune receptor and the microbiome-dependent environment. Most importantly, SCFAs produced in the gut were found to regulate immune responses in the lung, since GPR43-deficient mice also displayed a more inflammatory allergic response during experimental asthma.¹⁹ By feeding mice with fermentable fibers, the composition of both the gut and lung microbiota changed (in particular the ratio of Firmicutes and Bacteroidetes phyla), leading to an increase in the levels of circulating SCFAs that conferred protection against house dust mite-induced allergic pulmonary inflammation.⁹³ In particular, the SCFA propionate endorsed hematopoiesis and led to the generation of a population of lung DCs with enhanced phagocytic capacity and less capable of priming pathogenic Th2 responses. Contrary to what is mentioned above, these effects were mediated by GPR41 and not GPR43, thus pointing to intricate immunological specificities in the recognition of SCFAs. This is further highlighted by

the recent finding that another SCFA, butyrate, is consumed by colonocytes in the gut to protect intestinal progenitor cells, therefore disclosing a mechanism contributing to the maintenance of intestinal anatomy and homeostasis.⁹⁴

Another metabolic pathway whereby tryptophan metabolites produced by *Lactobacillus reuteri* in the gut microbiota regulate mucosal immunity has also been identified.⁸¹ The tryptophan derivative produced, indole-3-aldehyde, acts as an aryl hydrocarbon receptor ligand that contributes to the expression of IL-22 and allows commensal colonization by *C. albicans*. Of note, the tryptophan metabolism and downstream production of IL-22 were also found to regulate tolerance to *C. albicans* in the vaginal microbiome and functional genetic variants affecting these pathways were associated with the development of recurrent vulvovaginal candidiasis.⁹⁵ Whether tryptophan metabolites also display systemic effects remains to be elucidated. In any case, *Lactobacillus* spp., including *L. reuteri* but also *L. rhamnosus*, have been found to dampen allergic airway responses by inducing the expansion of Tregs.^{96,97} In addition, tryptophan catabolism was found to be required for a proper Th1/Treg balance and lung homeostasis in mouse models of CF.⁹⁸ Altogether, these data provide evidence about the role of the gut microbiome in regulating antifungal immunity in the lung and that manipulating SCFAs or Treg function might be a therapeutic option for fungal-induced allergic pulmonary inflammation.

In the CF lung, *P. aeruginosa* is able to produce metabolites with important consequences in its interaction with *A. fumigatus*.⁵⁸ Recently, molecular networking-based metabolomics revealed that the chemical makeup of the CF sputa comprises xenobiotics, specialized metabolites from *P. aeruginosa* and host sphingolipids.⁹⁹ Importantly, the microbial metabolites did not match those produced by laboratory cultures. For example, the quinolone signal from *P. aeruginosa* was readily detectable from cultured strains, but absent from sputum, even when its precursor molecules were present, thus suggesting that the metabolism of *P. aeruginosa* *in vivo* critically relies on signals provided by the chemical nature of the CF lung environment. As for the host sphingolipids, these contain the inflammatory mediator ceramide and may therefore have a potential role in the perpetuation of inflammation in CF. Importantly, facultative anaerobes such as *P. aeruginosa* are able to reduce the nitrogen in the reactive species released by inflammatory cells and use it to thrive within the chronically inflamed airways.¹⁰⁰

The volatile fraction of the metabolome, composed by volatile organic compounds (VOCs) is also critical for the understanding of the pathogenesis of respiratory diseases. In this regard, it was recently shown that *P.*

aeruginosa and *A. fumigatus* can interact not only directly, but at a distance via volatile-mediated communication.¹⁰¹ VOCs produced by *P. aeruginosa*, including dimethyl sulphide, dimethyl disulphide, 2,5-dimethylpyrazine and others were found to promote the growth of *A. fumigatus*, suggesting that the fungus requires sulfur uptake, provided at least in part, by exploiting the metabolism of *P. aeruginosa*. On the other hand, VOCs such as camphene, α - and β -pinene, and limonene, and the sesquiterpene compounds α - and β -trans-bergamotene, are specifically produced by *A. fumigatus* and can be distinguished from other pathogenic aspergilli, thus representing a novel, non-invasive, breath-based diagnostic approach.¹⁰²

Another important step toward a better understanding of the interaction between the microbiome and its host was recently provided by the association between the faecal levels of several secreted proteins, including the human β -defensin-2, calprotectin and chromogranin A, with microbial composition, diversity and functional richness.¹⁰³ Thus, not only do microbiota-derived metabolites regulate the immune system, but signals stemming from the host are also important in defining the homeostatic balance of the bacterial communities in the gut. This provides support to the notion that disruption of this dichotomy in either side might be detrimental for the activation of protective immune responses and may underlie susceptibility to disease.

Translating the microbiome-metabolome dialog into clinical application

Prompt and accurate diagnosis is crucial to a favorable outcome of respiratory fungal diseases, particularly IA. Although the introduction of molecular and serological diagnostic techniques into clinical practice has improved our diagnostic ability, considerable variability in performance still exists. Currently, the host-fungus interaction is being exploited to project more efficient and reliable fungal diagnostics¹⁰⁴ and efforts are being devoted to the implementation of clinical models aimed at the prediction of infection in high-risk patients.¹⁰⁵ Recent advances allowing us to collect more data on DNA sequences and metabolites are increasing our understanding of the relationship between the microbiota and associated metabolites at a whole-systems level. Determining the relative abundance of metabolically active bacteria and the metabolome composition during fungal infection is certain to contribute to the design of diagnostic strategies and tailored prescription of antifungals.

The post-engraftment expansion of Gammaproteobacteria in the gut of recipients of allogeneic hematopoietic stem-cell transplantation was found to be predictive of common

pulmonary complications and mortality,¹⁰⁶ raising the attractive possibility of using microbiota-derived information in the management of patients at high risk of fungal disease. The potential for clinical application of the microbiota was elegantly highlighted by the clinical trial that determined that treating recurrent *Clostridium difficile* infection using duodenal infusion of healthy donor feces was significantly more effective than the use of vancomycin.¹⁰⁷ In chronic respiratory diseases, such as COPD or CF, the manipulation of the lung microbiota could also be regarded as a valid adjunctive therapeutic strategy aimed at further improving airway clearance and the ability to restrict microbial migration and establishment in the lungs. Although the direct inhalation of probiotics may not be clinically viable, the strategic manipulation of microbiota-derived metabolites with immunomodulatory activity, antibiotics or quorum-sensing molecule inhibitors may be a potentially promising therapeutic option in the treatment of respiratory fungal diseases whose pathogenesis is associated, at least in part, with perturbations in the lung microbiota. In addition, several studies have described beneficial effects of enterically administered probiotics in the prevention of upper respiratory tract infections,¹⁰⁸ although it remains to be assessed whether benefit was conveyed via direct modification of the lung microbiota or indirectly via gut-mediated effects on systemic immune responses. Furthermore, by understanding the interaction between host genetics and microbiota composition in the context of fungal infection, the manipulation of the individual flora for a given host genome as a suitable therapeutic strategy is envisaged.

Conclusions

In this review, we have provided an up-to-date overview of the current knowledge on the role of the microbiota in the immunopathogenesis of respiratory fungal diseases, with a particular emphasis on aspergillosis. Increasing evidence supports a role for gut (and lung) dysbiosis in deregulated immune responses and inflammation, leading to the disruption of the balance between fungal colonization and overt disease. Although this is an active field of research, there is relatively limited overlap in the conclusions from different studies and few of them have directly related the described dysbiosis with a biological function in the pathogenesis of fungal disease. This limitation is mostly attributed to discrepancies between studies in terms of sampling procedures, sequencing approaches, and enrolment of patients with heterogeneous clinical traits. However, several bacterial populations have been shown to be involved in more than one clinical condition intrinsically associated with predisposition to fungal disease and these could serve as a starting point for the functional studies that are needed to make

the translation to new microbiome-based diagnostics and therapeutics in patients at risk. This will improve our insights into the various roles of the microbiota and its metabolic profiles and their potential as novel targets in respiratory fungal diseases.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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