

The potential impact of the pulmonary microbiome on immunopathogenesis of *Aspergillus*-related lung disease

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Aspergillosis is an infection or allergic response caused by fungi of the genus *Aspergillus*. The most common forms of aspergillosis are allergic bronchopulmonary aspergillosis, chronic pulmonary aspergillosis, and invasive pulmonary aspergillosis. *Aspergillus* also plays an important role in fungal sensitized asthma. Humans inhale *Aspergillus* spores every day and when the host is immunocompromised, *Aspergillus* spp. may cause severe pulmonary disease. There is increasing evidence that the microbiome plays a significant role in immune regulation, chronic inflammatory diseases, metabolism, and other physiological processes, including recovery from the effects of antibiotic treatment. Bacterial microbiome mediated resistance mechanisms probably play a major role in limiting fungal colonization of the lungs, and may therefore prevent humans from contracting *Aspergillus*-related diseases. In this perspective, we review this emerging area of research and discuss the role of the microbiome in aspergillosis, role of *Aspergillus* in the microbiome, and the influence of the microbiome on anti-*Aspergillus* host defense and its role in preventing aspergillosis.

Keywords: Allergic bronchopulmonary aspergillosis • Aspergillosis • Immune regulation • Mycobiome

Introduction

Aspergillus fumigatus is a ubiquitous fungal pathogen that releases thousands of conidia, more commonly known as spores, into the environment. After *Candida albicans*, *A. fumigatus* is the second most common cause of fungal infection in hospitalized patients. In healthy individuals, *Aspergillus* conidia are efficiently cleared by macrophages and neutrophils [1]. However, when the host is immunocompromised, such as neutropenia induced by

chemotherapy or via the chronic use of corticosteroids, inhalation of *Aspergillus* conidia (spores) can cause a spectrum of diseases such as invasive pulmonary aspergillosis (IPA) and chronic necrotizing pulmonary aspergillosis. Invasive aspergillosis is characterized by the transformation of conidia to hyphae (germination), which can invade the tissue. In addition to patients with severe immunodeficiency states, patients with chronic obstructive pulmonary disease (COPD) and influenza infection who are admitted to the ICU are at risk of developing IPA [2, 3]. Another disease manifestation in which *Aspergillus* plays a significant role is allergic airway disease. Patients with asthma or cystic fibrosis (CF) can develop an allergic disease in response to *Aspergillus*, with allergic bronchopulmonary aspergillosis (ABPA) being the

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most severe form. This other spectrum of disease caused by *Aspergillus* is not an infection, but rather an uncontrolled and detrimental inflammatory response to fungal colonization in the respiratory tract [4]. There is increasing evidence that the pulmonary microbiome plays a significant role in immune regulation, chronic inflammatory diseases, metabolism, and other physiological processes, and thus could influence the immune response in *Aspergillus*-related diseases by either contributing to or protecting from disease.

The pulmonary microbiome refers to all the microorganisms that inhabit the lungs. Classically, the lung was thought to be sterile, based on conventional culture methods [5]. However, recent DNA-based culture-independent techniques have enabled the detection and quantification of microbial species that cannot be cultured [6]. This has revealed that the lung actually comprises a relatively large number of bacterial taxa, and has increased our knowledge about the diversity, composition, and function of the pulmonary microbiome, although more comprehensive research may still be needed [5, 7]. To date, studies that have investigated the microbiome of the lung using culture-independent techniques are limited and the results are thus far inconclusive, mainly because of sampling techniques and the observation that the microbiome appears to be different in distinct regions of the airways [8]. In contrast to the oral and gut microbiome, studies on the pulmonary microbiome face challenges, since the lower respiratory tract is not easily accessible and does not contain a high microbial biomass [6]. These studies have been performed with small sample sizes, different populations, different sample collection techniques, different types of respiratory samples, and differences in 16S rRNA sequencing techniques, which makes it difficult to compare results. Furthermore, microbial communities in the lung are not equally distributed between lobes or even smaller segments, which might result in sampling errors. Accordingly, the potential impact of the pulmonary microbiome on the regulation of immunity of *Aspergillus*-related lung disease is an emerging field with many gaps in our mechanistic understanding.

The clinical spectrum of aspergillosis

IPA involves the invasion of the hyphae into the lung parenchyma, which causes hypoxia and bleeding in the airways and ultimately respiratory insufficiency [9]. This will cause fever, cough, and shortness of breath, and when *Aspergillus* hyphae invade the vascular wall, might also cause bleeding in the lung [9]. IPA has become a leading cause of death, mainly among hematologic and solid-organ transplant patients and the most clearly defined predisposing factor is a quantitative or qualitative defect in neutrophils. However, the increasing number of non-neutropenic immunocompromised patients diagnosed with IPA underlines the importance of non-neutrophil defense mechanisms as well [10, 11]. These include optimal NADPH-oxidase capacity in monocytes and macrophages and optimal interferon- γ (IFN- γ) responses [9].

IFN- γ has been shown to be important for protection against invasive aspergillosis by optimizing the phagocytic and killing capacity of neutrophils and monocytes [12]. NK cells as well as Th1 cells are important sources of IFN- γ and are thus considered to be important in the host defense against invasive aspergillosis [12].

Chronic pulmonary aspergillosis (CPA) is a disease state in which a significant load of *Aspergillus* is present in a preexisting cavity caused by tuberculosis or COPD. The growth of *Aspergillus* in this cavity is often limited by physiological barriers, however *Aspergillus* growth can become invasive and lead to necrosis of lung tissue, a disease called chronic necrotizing pulmonary aspergillosis [13]. It is likely that host defense mechanisms required to control CPA/or necrotizing CPA are similar to those needed to protect against IPA, such as intact neutrophil function and IFN- γ responses.

ABPA is currently the most severe allergic pulmonary complication caused by *Aspergillus* spp. It occurs in patients suffering from atopic asthma or CF. Pathological inflammatory responses in ABPA are mainly characterized by exaggerated Th2 responses, which are thought to be triggered by *Aspergillus*. The chemokine TARC (CCL17) has been shown to play an important role in both murine and human fungal sensitized asthma [14, 15]. CCL17 binds to its receptor CCR4 and induces Th2 responses that drive mast cell degeneration and increase IgE in the lung [16]. These inflammatory responses can result in poorly controllable asthma, bronchiectasis, and even fatal destruction of the lungs.

The gut microbiome and host immune responses

It has become apparent that changes in the gut microbiome influence host immune responses. This is exemplified in the development of spontaneous type 1 diabetes (T1D) in nonobese diabetic (NOD) mice, which has been shown to be dependent on the interaction between the innate immune system and gut microbiota [17]. NOD mice that are deficient in MyD88, part of the Toll-like receptor (TLR) signaling machinery in response to bacterial LPS, were protected against T1D. Notably, when these mice were housed in a germ-free environment, this protection was lost [17]. The composition of the gut flora of MyD88-deficient mice was shown to be responsible for the attenuation of the development of T1D in NOD mice [17]. Interleukin 1 (IL-1) receptor antagonist (IL-1Ra) mice normally develop spontaneous arthritis later in life. [18]. IL-1Ra KO mice can be protected from arthritis by housing under germ-free conditions, suggesting that the absence of the microbiome in the gut is responsible for the inflammatory conditions leading to arthritis in these IL-1Ra KO mice [18]. These data illustrate that in autoimmune diseases such as diabetes and arthritis, the gut microbiome can contribute to, as well as protect against, disease. This underscores the importance of a balanced microbiota and the complexity of its interaction with the immune system.

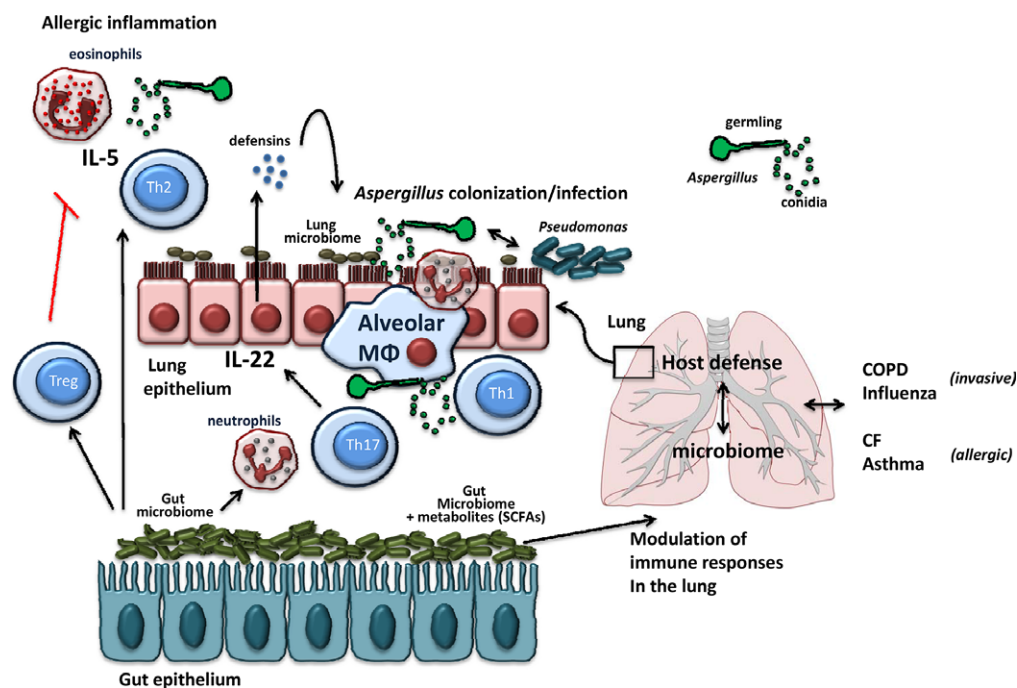


Figure 1. Interaction between the host microbiome and *Aspergillus*-induced immune responses. There is a dynamic interaction in the lung between the host defense and microbiome that can be influenced by diseases such as COPD and influenza that predispose for invasive aspergillosis, and cystic fibrosis (CF) and asthma, both of which predispose for *Aspergillus*-induced allergic inflammation (right). *Aspergillus* in the lung is sensed by alveolar macrophages, which recruit neutrophils and induce adaptive immune responses, such as Th1 and Th17 responses. *Aspergillus* is a potent inducer of the cytokine IL-22, which induces defensins, which could influence the composition of the pulmonary microbiome. Species such as *Pseudomonas* interact with *Aspergillus*, and when *Pseudomonas* is abundant in the pulmonary microbiome, *Aspergillus* colonization is often found (upper left). The gut microbiome and its metabolites, such as short-chain fatty acids (SCFAs), can modulate pulmonary host defense (lower right). In addition, the gut microbiome can enhance neutrophil killing, which is essential for *Aspergillus* host defense. Pulmonary Th2 responses induced by *Aspergillus* are influenced by the composition of the microbiome, and the gut microbiome plays an important role in modulating systemic Treg-cell function, which can inhibit pulmonary Th2 responses (left); mo: macrophage.

Despite this complexity in interaction, some studies have been able to identify specific interactions between the microbiome and host immune response. Short-chain fatty acids (SCFAs) are products of the intestinal microbiota and are recognized by the G-coupled protein receptor GPR43, a receptor that is mainly expressed on innate immune cells, such as neutrophils and activated macrophages. Mice lacking GPR43 were shown to be unable to resolve intestinal inflammation in an experimental colitis model, because of increased proinflammatory mediators, such as increased MPO production, and increased cell recruitment [19]. These data highlight that a specific host receptor can play a critical role in responding to a microbiome-dependent environment and demonstrates a molecular link between the host immune response and microbiome. Moreover, this study also underscored that the intestinal microbiome might not only influence host immune responses in the gut but could also have effects on distant organs such as the lungs, since GPR43-deficient mice also displayed a more inflammatory allergic response in a model of experimental asthma [5]. These data provide evidence that the intestinal microbiome may have an immunoregulatory function beyond the gut and potentially play a significant role in pulmonary diseases such as the various forms of aspergillosis (Fig. 1).

The role of the fungal microbiome on immune regulation

But what is the role of fungi as members of the microbiome, and are these fungi able to modulate immune responses? The total number of fungi in the microbiome is thought to be many degrees smaller than that of the bacterial microbiome and subsequently fungi belong to the “rare biosphere” (<0.1% of the total microbiome) [20]. This “rare biosphere” serves as a reservoir for potentially pathogenic microbes, such as *Aspergillus* and *Candida*, which are believed to grow out when the environment is disturbed or when the host is immunocompromised [20]. Nevertheless, this relatively small mycobiome could potentially contribute to disease. Mice lacking Dectin-1, which is an innate immune receptor that recognizes β -glucans present in the cell wall of fungi, showed an increased susceptibility to colitis, which was the result of altered responses to commensal fungi [21]. *Candida albicans* is a potent inducer of Th17 cells [22], and Th17 responses have been linked to autoimmune diseases such as multiple sclerosis and psoriasis [23]. When *Candida* cell wall components are present in the joints of an experimental arthritis model (streptococcus cell wall induced arthritis), their presence

in the joints can polarize the arthritis toward a more destructive phenotype, with destruction of cartilage and bone, which was Th17-driven [24]. These data suggest that fungal components such as β -glucans may indeed influence immune responses and contribute to autoimmune diseases such as colitis and rheumatoid arthritis. In addition, *Candida* induces prostaglandin E2 (PGE2), which contributes to Th17 responses [25]. In a recent report, it was shown that antibiotic treatment in mice altered the gut microbiome with outgrowth of commensal *Candida* species in the gut [26]. This was associated with increased plasma concentrations of PGE2, which induced M2 macrophage polarization in the lung [26]. Adoptive transfer of alveolar macrophages derived from antibiotic-treated mice increased allergic airway inflammation in the recipient mice, which in turn could be attenuated when these recipient mice were treated with PGE2 inhibitors [26]. Therefore, alteration in the gut microbiome favoring *Candida* outgrowth can have effects on polarization of alveolar macrophages and may contribute to allergic airway disease.

Ghannoum et al. recently characterized the mycobiome profile of the oral cavity in healthy individuals using culture-independent techniques, and found a total number of 85 fungal genera and 101 species [27]. The four most frequently obtained fungi were *Candida*, *Cladosporium*, *Aureobasidium*, and *Saccaro mycetales*. *Aspergillus* was present in 35% of the tested individuals. [27]. In addition, another study found that when the oral fungal microbiome was investigated in 40 healthy individuals, molds were recovered from all participants and yeast from 92.5% [28]. The most frequently isolated fungi were *Candida*, *Rhodotorula*, *Penicillium*, *Aspergillus*, and *Cladosporium* [28]. The fungal microbiome has also been analyzed in lung transplantation patients. Bronchoalveolar lavage of healthy subjects yielded minimal fungal amplification, whereas bronchoalveolar lavage from lung transplant patients showed moderate to high levels of fungal DNA, mainly consisting of *Candida* and *Aspergillus* [29]. Notably, *Aspergillus* can induce significant expression and production of the Th17 cytokine IL-22 [30]. IL-22 is known to induce antimicrobial peptides produced by epithelial cells, such as defensins that are important for host defense [31]. The induction of these antimicrobial peptides can have a significant impact on the microbial composition of the local microbiome [32]. Therefore, *Aspergillus* could modulate the local microbiome via immunological responses that are dependent on cytokines such as IL-22. However, in contrast to *Candida*, no studies to date have investigated the significance of *Aspergillus* as part of the microbiome regulating immune responses in disease settings.

Altered microbiomes in *Aspergillus*-related diseases?

The bacterial microbiome is likely to have effects on mycobiome composition either directly via bacterial–fungi interaction or indirectly via host responses. Mycelial colonization may become significant, and may eventually result in *Aspergillus*-related lung disease when the microbiological composition of the normal flora

in the respiratory tract is disturbed, such as in the case of an underlying disease, an altered immunological status of the host, or a decrease in ciliary activity of the mucous pulmonary epithelium. The underlying diseases that predispose to aspergillosis and their altered microbiome could thus provide significant insights into which alterations in the microbiome might predispose to aspergillosis.

Invasive pulmonary aspergillosis

Chronic obstructive pulmonary disease

Only recently, COPD has been recognized as a risk factor for IPA in critically ill patients admitted to the ICU [2, 33, 34]. COPD has not been associated with an obvious immunodeficient status, which might explain the increased susceptibility to IPA. Therefore, the role of the microbiome, or more specifically, the altered microbiome present in COPD and how it might play a role in predisposing COPD patients to IPA is of great interest. In the past few years, the airway microbiome in patients with COPD has been investigated mainly for its bacterial composition. These studies do not agree much regarding the composition and diversity of the pulmonary microbiome, which might be due to small sample sizes, different sampling techniques, and sites sampled [8, 35, 36]. Some retrospective studies have investigated the prevalence of *A. fumigatus* isolation from lower respiratory tract samples in COPD patients, using culture-dependent techniques [37–39]. In a large cohort of COPD patients who were admitted with severe exacerbation, *Aspergillus* was isolated in the sputum of 17% of the patients [40]. The concurrent isolation of other pathogens, especially *Pseudomonas aeruginosa*, was associated with a higher risk of *Aspergillus* isolation [26]. Length of stay and severity of disease were not associated with *Aspergillus* colonization, suggesting that the role of *Aspergillus* in COPD exacerbation appears to be minimal [40]. The question remains as to what extent the possibly altered microbiome in COPD contributes to the colonization of *Aspergillus* in COPD, and thus predisposes for IPA in COPD. The treatment of COPD, i.e. inhaled corticosteroids and bronchodilators, may contribute to the alteration of the microbiome [36] subsequently increasing the risk of *Aspergillus* colonization and infection. Additionally, tracheal intubation in critically ill, mechanically ventilated patients in general eliminates natural defense mechanisms such as the cough reflex, mucociliary clearance, and injures the tracheal epithelial surface, thereby providing direct access for bacteria and fungi from the upper into the lower respiratory tract [41].

Influenza

Another group of non-neutropenic patients with increased susceptibility to IPA are patients with influenza, specifically patients infected with H1N1 [3, 42–45]. Investigation of the microbiome in

patients with pneumonia, with and without H1N1, revealed that there was a relative abundance of *Firmicutes* and *Proteobacteria* in the lungs of patients with H1N1 [46]. More specifically, a number of *Pseudomonas* spp. were significantly increased in the H1N1-infected group. These bacterial species have a more prominent motility profile, and increased transcriptional regulation and metabolism, as well as an increased number of pathways involved in secondary metabolite biosynthesis and catabolism compared with that of genera found in the healthy lung [47], giving these species a competitive advantage. H1N1 infection therefore significantly changes the microbiome in the lung, which may contribute to secondary invasive infections including aspergillosis.

Allergic bronchopulmonary aspergillosis

Few studies to date have demonstrated a link between the microbiome and *Aspergillus*-related allergic airway disease or *Aspergillus*-sensitized asthma. An interesting study performed by Noverr et al. showed that alterations of the gut microbiome by antibiotic use predispose for *Aspergillus*-induced allergic airway disease in mice [48]. Mice were exposed intranasally to *A. fumigatus* conidia after antibiotic treatment or no antibiotic treatment. Although exposure of *Aspergillus* did not lead to invasive *Aspergillus* infection, an allergic inflammatory response characterized by increased Th2 cytokines and eosinophilia was observed in *Aspergillus*-exposed mice that received antibiotics [48]. These data may suggest that antibiotic exposure has an impact on the composition and diversity of the gut bacterial microbiome, which could alter the pulmonary immune responses to *Aspergillus* and result in allergic airway disease. It is likely that other fungi than *Aspergillus* contribute to fungal sensitized asthma as well, since the composition and extent of the fungal respiratory microbiome in patients with asthma is different from the fungi found in the sputum of healthy individuals, with *Psathyrella candolleana*, *Malassezia pachydermatis*, *Termitomyces clypeatus*, and *Grifola sor-dulenta* being more prevalent in the sputum of asthma patients [49]. However, besides providing fungal antigens, *Aspergillus* as part of the microbiome might also contribute to allergic airway inflammation via the production of lipoxygenase (LoxB), which shares homology with human 5-lipoxygenase, an enzyme known to be involved in the pathogenesis of asthma [50]. LoxB was shown to exacerbate acute pulmonary allergic responses [50]. Since the metabolic state of *Aspergillus* is determined by its micro-environment, local factors such as the microbiome might alter the metabolic state of *Aspergillus* and in this way predispose the patient to *Aspergillus*-induced allergic inflammation.

Cystic fibrosis

Although *A. fumigatus* colonization has been reported to be as high as 60% in CF patients, the incidence of invasive aspergillosis in these individuals is low [51, 52]. The main clinical manifestation of *Aspergillus*-related disease in CF patients is ABPA, which is characterized by a severe hypersensitivity reaction in

the lungs that occurs when the bronchi become colonized with *Aspergillus* spp. Recent studies on bacterial airway microbiota in CF patients revealed a predominant colonization of the airways with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenza*, and *Burkholderia cepacia* [53], indicating that a complex polymicrobial mixture of bacterial and fungal pathogens is present in the lungs of CF patients [52, 54, 55]. Within this unique environment, both bacteria and fungi might have the ability to form biofilms and interactions between these biofilms and pathogens may influence pathogenicity. Quorum sensing and cell-to-cell signaling play an important role in the competition between pathogens for space, nutrients, and persistence in the lungs of patients with CF. In particular, complex interactions between *Pseudomonas* and *Aspergillus* have been noted for their importance in the pathogenesis of CF [56]. Mowat et al. reported an antagonistic relationship between *A. fumigatus* and *P. aeruginosa* by direct contact and the release of small extracellular molecules. For example, the deletion of the principal quorum-sensing networks of *P. aeruginosa* significantly reduced the capacity for *A. fumigatus* to form hyphae and undergo biofilm development [57]. Interestingly, antibiotics targeting *Pseudomonas* spp. in CF patients resulted in a significant reduction of detection of *Aspergillus* in the sputum [58]. These data highlight the importance of the interaction between *Pseudomonas* spp. and *Aspergillus* in the pulmonary microenvironment [59]. Most likely, *Aspergillus* will also influence the complex nature of the polymicrobial flora in CF by its own metabolites. Thus, there is a complex and abnormal microbiome in CF, and a better understanding of the microbiome–*Aspergillus* interaction is likely to help to clarify why there is an increased prevalence of ABPA in CF patients.

Microbiome-mediated immune regulation and anti-*Aspergillus* host defense

Efficient anti-*Aspergillus* host defense is critically dependent on professional phagocytes, such as macrophages, monocytes, and neutrophils, and specific T-cell populations, such as Th17, Th1, and regulatory T (Treg) cells, which modulate the extent and nature of the immune response against *Aspergillus* [9]. Changes in the gut microbiome have a profound impact on local and systemic immune subsets, including T helper type (Th)1/Th2 balance, the development of Th17 and Treg cells, and natural killer (NK) T-cell function [60–63]. Since the gut microbiome can also influence mucosal sites of distant organs such as the lung, and the lymphocyte subsets at these distal sites play an important role in anti-*Aspergillus* host defense, it is likely that changes in the gut microbiome will influence anti-*Aspergillus* host defense as well [64]. Th17 cells have been shown to be detrimental and protective during IPA, indicating that Th17 responses in the lung need to be tightly balanced [65–69]. It was elegantly demonstrated that segmented filamentous bacterium (SFB) induces Th17 cells to produce IL-17 and IL-22 in the lamina propria [62]. These SFB might also be important for lung Th17 development, however in contrast to their participation in gut immunity, intestinal SFB were not able

to modulate the generation of pulmonary Th17 responses elicited in a (mouse) model of airway sensitization and antigen challenge [70]. It remains to be determined whether the lung microbiome also contains microorganisms with similar Th17-polarizing capacity as SFB in the gut, which would subsequently influence the anti-*Aspergillus* host response.

The increased susceptibility to invasive aspergillosis in patients with influenza suggests that influenza alters anti-*Aspergillus* host defenses, which might be via altering the microbiome as described earlier. In addition, the microbiome in the gut can influence the pulmonary host response against influenza, which was elegantly demonstrated in a mouse model of influenza infection [71]. Animals receiving antibiotic treatment before influenza infection mounted a significantly reduced antibody response to viral challenge, and these mice were incapable of inducing protective CD4⁺ T-cell-mediated IFN- γ [71]. This interplay between the gut microbiome and pulmonary host defense can thus result in a deficiency to induce Th1 responses. Th1 responses are essential for protective host defense against invasive aspergillosis, and the gut microbiome might thus also influence essential anti-*Aspergillus* host defense mechanisms.

Commensal bacteria in the gut have recently been shown to control the ability of phagocytic cells to rapidly produce type I IFNs systemically [72, 73]. The absence of commensal microbiota in the gut was shown to increase the susceptibility of germ-free mice to pulmonary viral infection [72, 73]. This was associated with decreased lymphocyte function, and intestinal colonization was required for optimal NK-cell cytotoxic activity, CD8⁺ T-cell clonal expansion, and virus-specific antibody production [73]. These data suggest that the gut microbiome can orchestrate pulmonary immune responses and influence type 1 IFN release by antigen-presenting cells (APCs) in the lung. Type 1 IFNs play an important role in anti-*Aspergillus* host defense [74]. It is therefore not unlikely that the gut microbiome can prime the anti-*Aspergillus* host response via modulating pulmonary DC type 1 IFN responses. In addition, peptidoglycan derived from gut bacteria can enhance neutrophil killing via NOD1 [75], and neutrophils are crucial for anti-*Aspergillus* host defense [76]. These data provide evidence that the gut microbiome has the potential to influence the anti-*Aspergillus* host response, making the gut microbiome an attractive target of study with the eventual goal to modulate host defense during invasive aspergillosis via manipulation of the gut microbiota.

The altered microbiome in *Aspergillus*-induced allergic pulmonary inflammation

Studies that investigated the role of the microbiome on *Aspergillus*-induced asthma have provided evidence that changes in the gut microbiome have the capacity to alter pulmonary Th2 responses. Infiltrates with lymphocytes and eosinophils are elevated in the airways of allergic germ-free mice compared with control SPF mice, which can be reversed by recolonization of GF mice

with the flora of SPF mice [77]. Moreover, when mice are treated with antibiotics, followed by a single oral gavage with *C. albicans*, the alterations of the gut microbiome drive the development of a CD4⁺ T-cell-mediated allergic airway response to *Aspergillus* without previous systemic antigen priming [48]. In this setting, *Aspergillus* induced increased levels of eosinophils, mast cells, IL-5, IL-13, immunoglobulin E, and mucus-secreting cells [48]. IL-22 has been shown to play an important role in an experimental model of *Aspergillus*-induced asthma [78]. Although IL-22 can be produced by Th17 cells, other cells such as invariant NK cells and innate lymphoid cells (ILCs) also produce IL-22 in significant amounts, which have been suggested to play an important role in regulating asthma [79]. ILCs are emerging as one of the critical immune cell populations that has the ability to orchestrate microbiome-mediated immune regulation in health and disease [80]. ILCs can express TLRs; ROR γ ⁺ ILCs express functional TLR2 [81]. Stimulation of TLR2 induced IL-2 production, subsequently resulting in increased expression of IL-22 in these ILCs [81]. Therefore, the microbiome might influence the inflammatory milieu directly via innate cells such as ILCs and enhance the allergic airway responses induced by *Aspergillus*.

Bacterial microbiome-mediated immune regulation in preventing *Aspergillus*-related disease

Instead of a pathological altered microbiome contributing to inflammation or an impaired host defense making the host more susceptible to *Aspergillus*-related diseases, one could also argue that the healthy microbiome actively protects against fungal overgrowth and maintains a balanced immune response that is beneficial to fighting infection. It has been shown that commensal bacterial derived signals can limit serum IgE levels. IgE can be potentially harmful since it can prime the responsiveness of basophil precursors in the BM that in turn can contribute to exaggerated allergic airway responses [82]. Interestingly, the observed increased IgE levels and increased basophils levels in the peripheral blood when mice were given oral antibiotics were dependent on B-cell intrinsic MyD88 expression [82]. Furthermore, metabolites produced by the microbiota can have important immunoregulatory functions, such as SCFAs. When mice were fed dietary fermentable fibers, the composition of the gut and lung microbiota changed (mainly the ratio of *Firmicutes* to *Bacteroidetes* was altered) and the concentration of circulating SCFAs increased, and these mice were protected against house dust mite induced allergic pulmonary inflammation [83]. Moreover, the treatment of mice with the SCFA propionate led to enhanced generation of macrophages and DCs in the BM and resulted in a lung DC population that had increased phagocytic capacity and was less capable in priming T cells toward Th2 responses [83]. These effects were not dependent on GPR43 as mentioned earlier, but on GPR41. Therefore, it is likely that alteration of the microbiome and its metabolites could contribute to preventing *Aspergillus*-related

diseases by increasing DC phagocytic capacity to fight invasive aspergillosis and by lowering the capability of DCs to prime for *Aspergillus*-induced allergic inflammation. The question arises as to whether protective immune responses, in the setting of *Aspergillus*-induced pulmonary allergic responses, could be induced by modulating the gut microbiome. Treg cells have been shown to attenuate Th2 responses. Indeed, it has been shown that oral *Lactobacillus rhamnosus* treatment in mice dampens allergic airway response, which is associated with an increase in Treg cells [84, 85]. This is further supported by studies that demonstrate that the oral administration of probiotic microbacteria in mice, such as *Bifidobacterium longum*, increases the number of Treg cells in the lung with subsequent attenuation of pulmonary allergic responses [86, 87]. These data provide evidence that the gut microbiome could regulate *Aspergillus*-driven Th2 responses in the lungs, and that increasing the amount of SCFAs or increasing Treg-cell function by manipulating the gut microbiome might prove to be a therapeutic option in *Aspergillus*-induced allergic pulmonary inflammation.

Conclusions and perspectives

It is likely that the composition of the microbiome and its effects on host immune responses, as well as the interaction of the microbiome with *Aspergillus* spp. are important determinants of the susceptibility to and severity of *Aspergillus*-related diseases. However, we are only at the beginning of exploring this new field of research as there are still challenges regarding standardized sampling of the lungs as well as the development of uniform sequencing techniques for the identification of the fungal pulmonary microbiome. Subsequently, many questions remain unanswered regarding the impact of the microbiome on immune regulatory networks that contribute to or protect against *Aspergillus*-related diseases. To what extent do metabolites such as SCFAs contribute to *Aspergillus*-sensitized asthma or ABPA, and could these metabolites, e.g., prime neutrophils for increased phagocytic capacity that is crucial for controlling invasive aspergillosis? What is the role of ILCs in *Aspergillus*-related diseases? And does the microbiome and its metabolites influence ILC function that can lead to protection or susceptibility to aspergillosis? Will the alteration of the microbiome and/or its metabolites in experimental settings protect against invasive or allergic aspergillosis? And could the pathways and receptors, such as the MyD88 pathway or the GPR43 or GPR41 that have been described as important pathways at the cross-section of the gut microbiome and immune response, also play a crucial role in aspergillosis? It can be anticipated that a better understanding of the complex interaction between the gut and pulmonary microbiome and the immune system will unravel new mechanistic insights in the pathogenesis of *Aspergillus*-related pulmonary diseases, and will lead to strategies that can target *Aspergillus* itself as well as the pulmonary immune system by influencing the host microbiome and/or its metabolites.

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Abbreviations: ABPA: allergic bronchopulmonary aspergillosis · CF: cystic fibrosis · COPD: chronic obstructive pulmonary disease · IL-1Ra: IL-1 receptor antagonist · ILC: innate lymphoid cell · IPA: invasive pulmonary aspergillosis · NOD: nonobese diabetic · PGE2: prostaglandin E2 · SFB: segmented filamentous bacterium · T1D: type 1 diabetes

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