We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Immunopathogenesis of Aspergillosis

Shreya Singh, Rimjhim Kanaujia
and Shivaprakash M. Rudramurthy

Abstract

Aspergillus species are ubiquitous saprophytes and opportunistic pathogens causing wide spectrum of diseases in humans depending on the host immune status. Following pathogen entry, various soluble bronchopulmonary factors enhance conidial clearance. However, due to virulence factors and poor host immune response *Aspergillus* conidia bind and damage the airway epithelium. The host immune cells like neutrophils and macrophages recognise *Aspergillus* spp. through various pathogen recognition receptors and form reactive oxygen species which mediate conidial killing. Neutrophils also attack extracellular hyphae by oxidative attack, non-oxidative granule proteins and neutrophil extracellular traps. In case of adaptive immunity, Th1 cells are crucial sources of IFN- γ mediated protective immunity. The Th17 also display a highly pro-inflammatory which is counterbalanced by a Treg cell. B cells and antibodies also enhance fungal clearance although excessive IgE production may result in atopy. The immune responses are influenced by changes in production of short-chain fatty acids by the gut microbiome which primes cells toward Th2 responses, and this is synchronized by the Innate lymphoid cells. This review provides comprehensive knowledge of various virulence factors of *Aspergillus*, antifungal host defences including innate and humoral immune response and regulation of host immunity by microbiome.

Keywords: Immunity, pathogenesis, aspergillus, genetic polymorphism, virulence

1. Introduction

Aspergillus species are globally ubiquitous saprophytes and are also opportunistic pathogens which have evolved in the environment and adapted to invade and proliferate within the human host. It can cause serious invasive infections. Invasive aspergillosis (IA) is associated with high mortality and morbidity which makes it essential to understand the factors involved in disease pathogenesis. The interplay between *Aspergillus* spp. and various components of the host immune system influences disease progression. Agent factors such as conidia size, temperature tolerance, hydrophobin /melanin expression etc. which contribute to virulence must be studied. Additionally, comprehensive knowledge of the host defenses, innate and humoral immune response, genetic susceptibility to *Aspergillus* and the role of microbiome in modulating immune response is important to study the disease immunopathogenesis.

In the genus *Aspergillus*, *Aspergillus fumigatus* is most commonly reported from human infections, followed by *A. flavus*, *A. terreus* and other uncommon species like *A. niger* and *A. nidulans* [1, 2]. It can cause plethora of infections, depending

on the immune status of the host as immunocompetent individuals with asthma or cystic fibrosis are predisposed to a hypersensitive response while Invasive aspergillosis (IA) is seen in severely immunocompromised patients.

A better understanding of the interplay between the host immune system and *Aspergillus* is important to understand disease pathology and can provide us with useful insights regarding potential therapeutic targets. In this review, we will thus discuss the pathogen related virulence factors, clinical spectrum of diseases caused by it, its interaction with various components of the host immune system, factors involved in regulating the anti-fungal immune response and will also give an overview of the genetic polymorphisms in immune pathways that predispose to aspergillosis. *Aspergillus* and disease pathology and progression are the result of both fungal growth and the host response.

2. Virulence factors

The various virulence factors involved in the pathogenesis of aspergillosis are summarized in Table 1.

	Function	Gene(s) involved	Reference
Enzymes			
Superoxide dismutases (SODs)	Oxidative stress defense	SOD genes	[3]
Protease	Degradation of host structural barriers		
1. Serine protease	Degrades elastin.	36-kDa	[4]
2. Metalloproteinase	Degrades fibrinogen and laminin.	23-kDa	[5]
3. Aspartic (acid) proteinase	Assist in host cell invasion of the hyphae.		[6, 7]
Catalase	ROS scavengers. Breakdown hydrogen peroxide (H ₂ O ₂) to oxygen and water.	catA - conidium-specific gene cat2 - mycelium-specific gene	[8, 9]
Toxins			
1. Gliotoxin	Inhibits macrophage phagocytosis.	18-kDa cytotoxin	[10, 11]
2. Restrictocin	Induces fragmentation and apoptosis of DNA in macrophages. Inhibition of T-cell activation.	gene cluster of aflatoxin biosynthesis regulated by <i>AflCDC14</i>	[12, 13]
3. Aflatoxin	RNA nuclease activity by cleavage of the phosphodiester bond in the 28S rRNA of eukaryotic ribosomes Induces DNA adducts causing genetic changes in cells responsible for carcinogenic potential <i>in vitro</i> . Also, epidemiologically to hepatocellular carcinoma.		[14]
Others			
1. Melanin	Masking of beta (1,3)-glucan. Delay macrophage activation. ROS scavengers.	pksP - polyketide synthase gene	[15, 16]
2. Rodlets	Rodlet proteins form hydrophobic layer around <i>Aspergillus</i> conidia and helps in its dispersal. ROS scavengers.	rodA gene	[17]

Table 1.
Virulence factors of *Aspergillus* species.

3. Risk factors and clinical spectrum

An elaborate range of diseases can be caused by *Aspergillus* species and the clinical spectrum depends on the immune status of the infected host. Correlation of clinical spectrum of aspergillosis and immune status in various condition has been depicted in **Figure 1**.

Immunocompetent Patient: In immunocompetent individuals *Aspergillus* spp. remain colonized as a saprophytic fungus. *Aspergillus* spp. can colonize in pre-existing cavities due to bronchiectasis, tuberculosis, cavitary neoplasia or sarcoidosis and cause chronic non-invasive infections like chronic pulmonary aspergillosis (CPA) [18, 19].

Hyper responsive or Atopic Patient: A hypersensitive response in these individuals in various forms like Allergic bronchopulmonary aspergillosis (ABPA), severe asthma with fungal sensitization (SAFS) and allergic rhinitis [20]. This is commonly seen in patients with cystic fibrosis (CF) and poorly controlled or steroid-refractory asthma [20]. In cases of CF, inflammation of bronchial mucosa and abnormal mucus can result in fungal colonization and up to 10% patients develop sensitization to *A. fumigatus* [21]. This can further progress to ABPA suggesting the importance of testing such patients with markers of immune hyper-reactivity.

Immunocompromised Patient: IA is a dreaded, life-threatening disease with a high mortality ranging from 40–80% [22, 23]. It is commonly seen in individuals with hematological malignancies such as acute leukemia; solid-organ and hematopoietic stem cell transplant patients; patients on prolonged corticosteroid or chemotherapy. Invasive pulmonary aspergillosis (IPA) is also reported in patients with history of influenza or coronavirus disease and those receiving broad-spectrum antibiotics [24, 25]. Genetic susceptibility to IA is also seen in patients

Figure 1.
Correction of clinical spectrum of Aspergillosis and immune status in various condition. *CID: Congenital immunodeficiency disorders includes chronic granulomatous disease, CARD9 deficiency, leukocyte adhesion deficiency, Job's syndrome, pulmonary alveolar proteinosis.

with congenital immune deficiencies like Caspase recruitment domain-containing protein-9 (CARD-9) deficiency and Chronic granulomatous disease [26, 27].

4. Pathogenesis

The range of ailments caused by *Aspergillus* depends on the host immune status. In atopic individuals the T helper 2 lymphocyte leads to hypersensitive response with increase in eosinophil counts and serum IgE levels. Formation of non-invasive aspergillomas is seen in CPA following repeated exposure to conidia in pre-existing cavitary lesions. IA is a destructive form of *Aspergillus*-related disease seen commonly in immunocompromised and critically ill patients.

5. Pathogen entry

The mode of reproduction in *Aspergillus* is predominantly asexual by formation of conidia (2–5 µm in size) which are ubiquitously present in the environment. These dormant conidia disperse in air easily due to their small size and common occurrence in soil, seeds and grains, decaying vegetation etc. and humans can inhale several hundred conidia per day. *Aspergillus* spp. are also found indoors in moisture damaged buildings both at homes and healthcare facilities [28]. There are therefore recommendations to avoid known sources of fungal proliferation (plants and flowers) in indoor places as they can serve as natural niches for fungal growth [29].

Conidia being small bypass the natural host nasal and bronchial defenses. The rodlet layer forms a hydrophobic layer outside conidia and protects it from host defenses and reach the lung alveoli. Natural defenses like mucociliary clearance and cough reflex are further compromised in intubated and mechanically ventilated patients. Also, the tracheal and bronchial epithelium is injured and provides easier passage for fungal conidia to the lower respiratory tract. Among healthy hosts, neutrophils and macrophages effectively clear the *Aspergillus* conidia. However, in immunocompromised patients, few conidia start swelling and become metabolically active after losing the outermost rodlet layer. These conidia, then germinate to produce fungal hyphae and cause a spectrum of invasive diseases.

6. Interaction with the innate immune system

The interaction of *Aspergillus* with cells of the innate immune system is depicted in **Figure 2**.

6.1 Soluble lung components

Various soluble factors found in the bronchopulmonary fluid are involved in *Aspergillus* defense including pathogen recognition receptors (PRRs) like C-type lectins, mannose binding ligand (MBL), Surfactant proteins (SP) – A and –D and pentraxin (PTX). These soluble factors enhance complement activation and phagocytosis of conidia, thus contributing to its clearance.

Although components of the complement system are predominant in serum they can also be found at lower levels in bronchial and alveolar fluid. Conidia and hyphae of *Aspergillus* species have been shown to bind to C3 followed by its cleavage to a ligand for phagocytic complement receptors iC3b. It has been reported

Figure 2.
Innate immune response to Aspergillus infection. The conidia of Aspergillus spp. are inhaled and enter the lung where they encounter various soluble lung components including antibodies, complement factors and anti-microbial compounds. Those conidia which swell and undergoes germination further interact with a variety of innate immune cells including alveolar macrophages, dendritic cells, and NK cells. Conidial germination and development of hyphal forms is also prevented by neutrophils.

that the common pathogens *A. fumigatus* and *A. flavus* bind to fewer C3 molecules compared to other species making their complement-mediated phagocytosis and killing, less effective [30]. Hyphae and conidia from various *Aspergillus* spp. bind to alternative complement receptors like complement inhibitor factor H and the factor H family protein FFHL-1 which prevents complement cascade activation thereby protecting the fungus [31]. *A. fumigatus* and *A. flavus* have also been seen to produce a soluble complement-inhibitory factor which inhibits the activation of the alternative complement pathway [32]. This also acts as a defense mechanism of these species contributing to their overall pathogenesis.

6.2 Respiratory epithelial cells

The airway epithelial cells are the first cells to encounter inhaled *Aspergillus* conidia, which bind to it via sialic acid residues and subsequently modulate it. Other conidial proteins also mediate binding to fibrinogen, laminin and fibronectin which are all linked with lung injury indicating a role in adhesion and colonization [30]. A broad range of antimicrobial peptides of the defensin family are produced by the respiratory epithelial cells. Although the contribution of airway epithelial cells is less robust than that of the alveolar macrophages and germinating conidia and hyphae of *Aspergillus* are recognized by various PRRs on epithelial cells and subsequently assist in initiating pro-inflammatory response.

The proteases secreted by *A. fumigatus* cause desquamation and shrinkage of the respiratory epithelial cells along with actin cytoskeletal rearrangement with loss of cellular attachment and focal contact, thus assisting in invasion by germinating hyphae [33]. Secondary metabolites like gliotoxin, fumagillin, helvolic acid, verruculogen also damage airway epithelium and interfere with mucociliary clearance [30, 34].

6.3 Pathogen recognition by innate immune cells

The recognition of *Aspergillus* by host immune cells is mostly via the PRRs – TLR1, TLR2, TLR4, TLR6 and the C-type lectin receptor i.e. dectin-1 [35]. TLR2 recognizes both hyphal and conidial form, while TLR4 recognizes only the hyphal morphology [36, 37]. The protective role of TLR4 mediated immune recognition has been seen in allogeneic hematopoietic stem cell transplant patients where it is observed that TLR4 polymorphisms are associated with IA [38]. The critical role of TLR6 in regulation of allergic inflammatory response in chronic fungal-induced asthma was studied by Moreira et al. in mice and the absence of TLR6 was found to be associated with less production of IL-23 and Th17 responses causing exacerbation of asthma [39]. Interestingly, the inflammatory response to *A. fumigatus* is intact in alveolar macrophages even in the setting of TLR2 deficiency and mice with defects in TLR2/TLR4 or its downstream effectors (like MyD88) have higher susceptibility to *A. fumigatus* lung infection, only in the setting of neutropenia [40–42].

Dectin – 1 is also an important PRR recognizing beta (1,3)-glucan on *Aspergillus* in both immunosuppressed and immunocompetent hosts. Although beta (1,3)-glucan is usually masked by the rodlet layer on resting conidia, the conidial swelling on entry in host epithelium exposes it, causing dectin – 1 mediated recognition and phagocytosis. Macrophages stimulation by *A. fumigatus* conidia increases intracellular PRR expression as well eg. Nucleotide-binding oligomerization domain (NOD) proteins ((NOD1 and NOD2) followed by production of proinflammatory cytokines which contribute to innate immune response [43].

6.4 Alveolar macrophages

Alveolar macrophages recognize and phagocytose fungal (1,3)-glucan bound to dectin-1. Internalization of conidia occurs within 2 hours and then conidial swelling begins [44]. This is an important requirement for induction of reactive oxygen species (ROS) production by the macrophage. Kinetic studies indicate that maximum ROS production occurs after 3 hours of phagocytosis resulting in fungistatic inhibition of germ tube formation due to which conidia are unable to germinate [44]. In immunosuppressed mice, although corticosteroid intake does not directly affect the internalization of conidia by alveolar macrophages there is impaired killing of *A. fumigatus* conidia due to defective production of ROS thereby increasing susceptibility to IA [44, 45]. The exact mechanisms of conidial killing by ROS are unknown and could be via direct toxicity or by acting as a cofactor for other phagolysosomal toxic molecules like elastase, cathepsins, proteases and chitinases [46]. In addition to phagolysosome acidification, phosphatidylinositol (PI) 3-kinase activity is also an important requirement for proper killing of conidia [47].

Neutrophils and macrophages produce nitric oxide (NO) and reactive nitrogen intermediates (RNI) that can also contribute to conidial killing. However, the expression of nitrogen oxidative species (NOS) which is seen in classically activated or M1 macrophages does not have much effect on conidial killing. A study by Lapp et al., reported that in *A. fumigatus* genes encoding flavohemoglobins (*FhpA* and

FhpB) which converts NO to nitrate and S-nitrosogluthathione reductase (*GnoA*) which reduce S-nitrosogluthathione to ammonium and glutathione disulphide are observed [48]. Although, these genes play a major role in detoxification of host derived RNI, they were not found to be essential for virulence.

Following macrophage phagocytosis, dihydroxynaphthalene-melanin (DHN-melanin) of *A. fumigatus* prevents the phagolysosome acidification allowing conidial germination. However, *A. terreus* conidia lack the genes for DHN-melanin synthesis and instead produce a different type of melanin, i.e., Asp-melanin [49]. Although Asp-melanin does not impede acidification of phagolysosome it hampers phagocytosis and contributes to the survival and long-term persistence of *A. terreus* even in acidic environment.

In a study by Bhatia et al., alveolar macrophages were found to express Arginase 1 (Arg1) a key marker of alternatively activated macrophages (AAMs)/M2 macrophages after infection by *A. fumigatus* [50]. These macrophages efficiently phagocytose conidia and play a crucial role in pathogen clearance. The activation of macrophages is also followed by translocation of mitogen-activated protein kinases (MAPKs) to the nucleus where they phosphorylate the transcription factor NF-kappa B, thus activating a pro-inflammatory immune response.

6.5 Neutrophils

Neutrophils are professional phagocytes playing a pivotal role in innate immunity. Neutrophil recruitment is essential for effective *Aspergillus* clearing as they attack the germinating conidia and extracellular hyphae which have escaped macrophage surveillance. Neutrophils utilize TLR2, TLR4 and dectin-1, to identify and respond to *Aspergillus*. It can also be recognized directly by the complement receptor 3 (CD3, i.e., CD 11b/CD18), antigen-antibody complex detection by the Fcγ receptors (FcγR) or indirectly by opsonisation by various soluble components in lung environment.

In a study by Braem et al., higher deposition of the serum C3b was reported on germ tubes and swollen conidia compared to dormant conidia [51]. Also, patchy deposition of both C3b and immunoglobulin G (IgG) is seen over dormant conidia compared to uniform deposits on other morphotypes.

The release of chemotactic molecules, like C5a, increases migration of neutrophil to the infection site. The soluble mammalian extracellular β-galactose-binding lectin, galectin-3 is released in infected host tissues and facilitates neutrophil recruitment to the site of *A. fumigatus* infection by directly stimulating neutrophil motility in addition to exhibiting with both antimicrobial and immunomodulatory activities [52].

Neutrophil mediated killing involves both oxidative killing by NADPH oxidase which generates superoxide and myeloperoxidase and non-oxidative granule proteins containing various compounds with antimicrobial activity e.g., defensins, serine proteases, lysozyme, pentraxin-3 and lactoferrin [53]. Neutrophils attach to hyphae, spread over their surfaces, and degranulate thereby damaging the fungal hyphae. Neutrophils form aggregates in the lung and restrict conidial germination via lactoferrin mediated sequestration of iron [54]. Also, neutrophils produce lipocalin-1, which sequesters fungal siderophores thereby inhibiting fungal growth [55].

Another neutrophil dependent defense is the formation of neutrophil extracellular traps (NETs). Conidia and germ tubes of the *A. fumigatus* have been shown to trigger the formation of NETs. Pathogens in contact with the NETs become immobilized, limiting the spread of the infection. Calprotectin, a chelator of Zn^{2+} and Mn^{2+} ions is also produced by neutrophils and is associated with the *Aspergillus*-induced

NETs [56, 57]. Thus, in view of the important role that neutrophils play against *Aspergillus*, it is no surprise that patients with qualitative or quantitative defects in the neutrophils experience a greater risk of IA. It is worth mentioning however, that neutrophils may act as double-edged swords, since these are needed for fungal eradication but can also cause further lung injury by release of proteases and ROS. Thus, stringent regulatory mechanisms are essential to balance the protective activity and immunopathological responses for efficient control of the *Aspergillus*.

6.6 Natural killer cells

There is growing evidence suggesting the role of NK cells in immune response against *Aspergillus* spp. Direct antifungal activity via cytotoxic molecules like perforin and NK cell derived cytokines and interferon modulate the activation of other immune cells. *A. fumigatus* activates NK cells resulting in the production of low-levels of TNF- α , IFN- γ and lytic granules and release of fungal DNA [58]. These cells are a major source of early IFN-gamma production in the lungs of neutropenic patient with IA causing higher expression of IFN-inducible chemokines and subsequently enhancing macrophage antimicrobial effects. Studies in mice-models also suggest a critical role of NK cells in the pulmonary clearance of *A. fumigatus* [59].

Interestingly, in a study by Santiago et al., down-regulation of NK cell activating receptors NKG2D and NKp46 and a failure of full granule release was observed on contact of NK cells with *A. fumigatus* hyphae [59]. They also reported *A. fumigatus*-mediated NK cell immune-paresis which reduces cytokine-mediated response causing immune evasion during pulmonary aspergillosis [59]. Characterization of the clinical impact of NK cells in antifungal host immune response is still in its nascent stage as it involves complex interplay between multiple arms of the immune system [60].

6.7 Dendritic cells

Dendritic cells (DCs) bridge the innate and adaptive immune responses. They not only sense and patrol the lung environment but also initiate host response by antigen presentation which primes the T cell responses and causes cytokine secretion. Immature DCs are phagocytic and constantly perform surveillance of the lung environment while expressing PRRs like TLR 1, 2, 3, 4, 6 and Dectin-1 on cell surface that recognize various pathogen-associated molecular patterns (PAMPs). After phagocytosis, *A. fumigatus* conidia have been reported to escape from DCs, whereas some species like *A. terreus* persist with long-term survival, protecting them from anti-fungal action [49].

Typically, DCs are of two types, the plasmacytoid (pDCs) which are IFN α (type I interferon)-producing cells with a significant role in antifungal response and Classical (cDCs) which remain in the lymphoid tissue and cross-present antigens to T cells [61]. There is considerable plasticity in the functional activity of pulmonary DCs depending on the morphology of invading fungus [62].

1. Although DCs internalize both conidial and hyphal form of *A. fumigatus*, internalization of conidia occurs by coiling phagocytosis while entry of hyphae occurs by zipper-type phagocytosis. Also, phagocytosis of conidia is via involvement of a C-type lectin receptor while CR3 together with Fc γ R mediate the entry of opsonized hyphae.
2. Cytokine production is also variable depending on the fungal morphotype as TNF- α response is seen to any fungal form, but IL-12 is produced on exposure to conidia, while IL-4/IL-10 upon phagocytosis of hyphae.

- 3. The pulmonary DC transport *Aspergillus* fungal forms to the draining lymph nodes and spleen followed by functional maturation and eventual degradation for efficient antigen presentation.
- 4. The DCs also direct both local and peripheral T helper cell in response to fungus.

7. Interaction with the adaptive immune system

The adaptive immune response to *Aspergillus* infection is depicted in Figure 3.

7.1 Role of T-cells

Antigen-specific Th1 cells are crucial sources of IFN- γ mediated protective immunity to *A. fumigatus* [18, 58]. Peripheral blood of healthy adult donors has been found to have *A. fumigatus* specific effector/memory CD4 T cells with Th1 phenotype [63, 64]. A Th17 phenotype is noted in lung-derived *Aspergillus*-specific T cells [65]. IL-22 is produced by Th17 cells and has shown to play a crucial role in regulating *Aspergillus* induced asthma [66]. Like neutrophils, Th17 responses represent a “double-edged sword”. During pulmonary fungal infections, the Th17 cell usually display a highly pro-inflammatory profile, which is detrimental to the infected host. The Th2 cell-mediated immune responses along with Th1 and Th17 induces chronic pulmonary inflammation and lead to significant lung damage [67, 68]. This

Figure 3. Adaptive immune response to aspergillus infection. *Aspergillus spp.* antigens are presented to naive T cells in peripheral lymphoid organs by dendritic cells and macrophages which further induces inflammation with coevolution of Th1, Th2, and Th17 response. B cells are also stimulated resulting in formation of anti-fungal antibody producing plasma cells.

allows influx of macrophages followed by differentiation of both M1 and M2 subtypes [69]. These macrophages and T cells play a key role subsequently promoting extensive remodeling of medium- and small-sized pulmonary arteries. Pulmonary artery pathology including an increase in intimal area, smooth muscle proliferation, calcification of elastic membrane, and narrowed arterial lumens is seen in those with fatal asthma [70].

In healthy subjects, a strong Treg response has been seen as a part of the normal physiological T-cell repertoire which counterbalances the *A. fumigatus* specific T cells [71]. This intriguing finding raises the possibility that colonizing *A. fumigatus* may selectively promote Treg responses and subsequently limit antifungal immune activity. Activation of indoleamine 2,3- dioxygenase (IDO) as a regulator of infection-linked tissue pathology is now being recognized as it acts via local tryptophan depletion, or generation of immunomodulatory metabolites. Interaction of TLRs with PAMPs induces IDO which regulates the inflammatory/anti-inflammatory status of the innate immune cell and modifies the local tissue microenvironment. There is also activation of GCN2, a T-cell stress-response kinase which senses amino acid starvation and impairs lymphocyte proliferation while enhancing polarization toward a Treg phenotype [72]. In patients of CF with ABPA, dysregulation of the IDO pathway is seen at both the genetic and transcriptional levels, leading to an imbalanced Th17/Treg with high Th2 polarization resulting in chronic inflammation and significant lung damage in response to *A. fumigatus* [73].

7.2 Role of B-cells

In a study by Montagnoli et al., the role of B cells and antibodies in the generation of antifungal immune resistance was studied in B cell-deficient (μ MT) mice which were infected with *A. fumigatus* [74]. They reported that, although passive transfer of antibodies helped in fungal clearance, a compensatory increase in both innate and Th1-mediated resistance to infection was seen in μ MT mice with aspergillosis. This suggests that in the absence of opsonizing antifungal antibodies, the nature of the interaction between the innate immune cells and with fungi may be modified with subsequent development of long-lasting antifungal immunity [74].

Chen et al., demonstrated that basophil interaction with IgD bound antigens and activation of TLRs induces expression of B-cell-activating factor (BAFF), an important regulator of B-cell activation, proliferation, and immunoglobulin production. This results in IgG and IgE production by B cells, pointing to a role of basophils in adaptive immune responses [75]. In a study by Boita et al. stimulation of basophil membrane by *Aspergillus* resulted in upregulation of BAFF expression in patients with SAFS and ABPA. These patients had high IgE suggesting the role of basophils in polyclonal IgE production [76].

8. Role of the microbiome

Host immune responses are influenced by changes in the gut microbiome. Short-chain fatty acids (SCFAs) produced by the gut microbiome are recognized by innate immune cells like macrophages and neutrophils expressing G-coupled protein receptor GPR43 [77]. The gut microbiome also plays a crucial role in anti-*Aspergillus* host defense by coordinating lymphocyte subsets at the mucosal level in distant organs such as the lungs. Although, fungal microbiome compromise <0.1% of total microbiome, fungal cell components such as β -glucans may influence immune responses as perceived by their role in autoimmune diseases [78]. *In-vivo* studies in mice have revealed that intake of SCFA (propionate/butyrate) or supplementation

of diet with fermentable fibers which increases SCFA producing bacteria, increases the generation of DCs and macrophages in the lung and bone marrow with increased phagocytic capacity [79–81]. These alterations also reduce the ability to prime cells toward Th2 responses lowering DC ability to induce *Aspergillus*-allergic inflammation [82].

The intestinal segmented filamentous bacterium (SFB) have been shown to induce Th17 cells producing IL-17 and IL-22 in the lamina propria of the gut and can even regulate pulmonary adaptive immune response by increasing Th17 responses in the lung [83, 84]. However, it is important to determine whether lung microbiome also has similar Th17-polarizing ability which can influence anti-*Aspergillus* host response.

It has also been observed that in germ-free mice, the absence of commensal gut microbiota leads to increase susceptibility to pulmonary viral infections. Hence, the gut microbiome can influence pulmonary immune responses by release of type 1 IFN [85, 86]. Intestinal colonization of microorganism is necessary for cytotoxic activity by NK-cell, CD8⁺ T-cell clonal expansion, and production of specific antibodies [85].

Recently, innate lymphoid cells (ILCs) have emerged as an important cell population that has the capacity to synchronize microbiome-related immune regulation [87]. ILCs can express functional TLR2 which on stimulation induces IL-2 production, subsequently increasing the expression of IL-22, enhancing the allergic airway responses induced by *Aspergillus* spp [88]. It has also been observed that commensal bacterial limit the production of serum IgE levels which directly influences bone marrow - basophil precursors, leading to increased allergic airway responses [89].

The treatment of diseases like COPD with steroids and bronchodilators, may also alter the microbiome [90] which can subsequently increase the risk of colonization and infection by *Aspergillus* spp. In patients with Influenza, significant changes in the lung microbiome have been observed with a relative abundance of *Firmicutes* and *Proteobacteria* more specifically, *Pseudomonas* spp., which contributes to secondary invasive infections by *Aspergillus* spp. [91, 92]. Other factors like antibiotic exposure can also influence the micro-environment of the microbiome, which can affect the pulmonary immune responses to *Aspergillus* causing allergic airway diseases [93]. In patients with CF, interaction between fungal and bacterial pathogens and their biofilms may influence pathogenicity which can be observed by significant decrease in *Aspergillus* in the sputum on treatment with anti-pseudomonal antibiotics [94, 95].

9. Genetic susceptibility to aspergillosis

The genetic polymorphisms within pattern recognition receptors PRRs (*TLR1*, *TLR2*, *TLR4*, *TLR5*, *TLR6*, *TLR9*, *Dectin-1*, *Dectin-2*, *DC-SIGN*, *MASP*, *MBL*, *PTX-3* surfactant protein-A2 and plasminogen) cytokines (*IL1*, *IL10*, IFN- γ , *CXCL10*, *ARNT2*,) and their receptors (*CX3CR1* and *IL-4R α*) is depicted in –Table 2.

10. Conclusion

The clinical spectrum of *Aspergillus* related infections depends on the host immune status ranging from allergic manifestations in immunocompetent atopic individuals to invasive disease in immunosuppressed individuals. Various components of the innate and adaptive immune system form an intricate network modulating host response to *Aspergillus* exposure. Many future studies are required to study

Gene	Function	SNP position	Disease condition	Reference
Pattern Recognition receptors (PRRs)				
TLR1	TLR1 forms heterodimer with TLR2 and facilitate the fungicidal activity by various oxidative pathways	239 C/G [80 R/T] 743 A/G [248 S/N] 1063 A/G	IA	[96]
TLR 2	TLR-2 act as PRR for <i>Aspergillus</i> spp. Antigens and activate innate immune cells. Further downstream signaling via TLR2 promote the fungicidal activity by various oxidative pathways which lead to proinflammatory cytokines release.	Arg753Gln (G + 2258A) polymorphism affects the TIR domain of TLR-2 and impairs its functional activity.	IA	[97]
TLR4	TLR4 promotes fungicidal activity	[299 D/G] 1363 C/T [399 I/T] 1063 A/G [299 D/G]	IA after HSCT [EORTC] CCPA	[38, 98, 99]
TLR5	TLR-5 induction causes increase in expression of pro-inflammatory cytokines	1174C T (STOP codon)	IA	[100]
TLR6	It promotes IL-23 release and a subsequent Th17 response.	745 C/T [249 S/P]	IA after HSCT [EORTC]	[96]
TLR9	It recognizes unmethylated CpG DNA and induces innate immune responses.	1237 C/T [Promotor]	ABPA	[98] [101]
<i>Dectin-1</i>	Dectin-1 is act as a PRR, which is present on myeloid cells surface and expressed by DCs and macrophages. It is specialized for recognition of β -1,3-glucan of fungal species. It leads to production of chemokines and cytokines and causes recruitment of neutrophil recruitment and ROS production.	Y238X polymorphism [Stop Codon Polymorphism]	IA	[102] [103] [104]
<i>Dectin-2</i>	Dectin-1 is act as a PRR, which is present on plasmacytoid dendritic cells (pDCs). It is specialized for recognition of α -mannans of fungal species. It leads to cytokine production, extracellular trap (pET) formation and ROS production.	(CLEC6A – A/G) [Intron] (CLEC6A - C/T) [Intron]	IPA	[104]
<i>DC-SIGN</i>	DC-SIGN is a CLR. It recognizes galactomannans.	336 A/G [promoter] c.898 A/G [3'-UTR] c.74928 C/T [3'-UTR] IVS2 + 11 G/C [Intron]	IPA	[104]

Gene	Function	SNP position	Disease condition	Reference
pentraxin (PTX3)	PTX3 is a soluble opsonin. It is produced by phagocytes that facilitates microbial recognition and phagocytosis of conidia.	+281A/G [Intron 1] +734A/C (D48A) [Exon 2] +1449A/G [Intron 2]	IA	[105] [106]
Mannose-binding lectin-associated serine protease (MASP2)	MASP binds directly to <i>Aspergillus fumigatus</i> and promote complement activation and phagocytosis	380 A/C [D120G]	IA	[107]
MBL	MBL is a soluble PRR. It opsonizes the carbohydrate moieties of fungus and activates the lectin complement pathway using the MASPs and induces the release of proinflammatory cytokines.	868 C/T [52 C/R] 1011 A/G [Intron] 868 C/T [52 C/R]	CCPA ABPA CNPA	[108–113]
Plg	Plasminogen is produced by phagocytes that facilitates microbial recognition.	28904 A/G ^a [472 N/D]	IA	[114–116]
SFTPA2 surfactant protein-A2		1660 A/G [94 R/R] 1649 C/G [91 A/P] 1492 C/T [Intron]	ABPA	[117, 118]
Cytokines				
CXCL10	It is an ‘inflammatory’ chemokine. It binds to CXCR3 and mediate leukocytes recruitment such as eosinophils, T cells, NK cells and monocytes.	11101 C/Ta [Downstream] 1642 C/Ga [3’ UTR] 1101 A/Ga [Promotor]	IA	[119] [120] [121]
ARNT2	It regulates the activity and differentiation of phagocytic cells like macrophages and lymphocytes.	80732053 [Intron]	IA	[122]
IFN- γ	It promotes differentiation of Th1 response	1616 C/T ^a [Promotor] 1082 A/G [Promotor]	IA	[123]
IL-10	IL-10 plays a significant role in the development of atopy. It inhibits the activity of Th1 cells, NK cells, and macrophages which are essential for clearance of fungus.	2068 C/G ^a [Intron] 1082 A/G [Promotor] 1082 A/G – 819 C/T – 592 A/C [Promotor] 1082 A/G [Promotor]	IA ABPA	[124] [125] [126]
IL-4R alpha	IL-4 released by T cells binds to the IL-4 receptor (IL-4R) on B cells resulting in B cell proliferation and IgE isotype switching.	4679 A/C/G/T [75 I/L/F/V]	ABPA	[127]
Cytokine’s receptors				
TNFR2 TNF receptor type 2	TNFR2 (p75) receptor is expressed by T regulatory cells for survival during clonal expansion.	322 [Promotor]	IPA	[107]

Gene	Function	SNP position	Disease condition	Reference
Interferon regulatory factor - 4 (IRF4)	It regulates the NFκB pathway and cell proliferation and modulates the differentiation of different DC and Th17-mediated immune responses against <i>Aspergillus fumigatus</i> .	rs12203592	IA	[128]
CX3CR1	Modulates the interaction of fungal pathogens with immune phagocytes.	39286825 [Intron] 39293757 [Intron]	IA	[122]

TLR-Toll-like receptor, IL – Interleukin, PRR – Pathogen Recognition Receptor, Th – T helper cells, DC-SIGN - Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin, PTX3- Pentraxin, MASP2 - Mannose-binding lectin-associated serine protease, MBL - Mannose-binding lectin, CXCL - chemokine (C-X-C motif) ligand, ARNT2 - Aryl hydrocarbon receptor nuclear translocator 2, IL-4R alpha - Interleukin 4 receptor alpha, TNFR2 - TNF receptor type 2, IRF4 Interferon regulatory factor - 4, CX3CR1 - CX3C chemokine receptor 1, IA- invasive aspergillosis, IPA- invasive pulmonary aspergillosis, CCPA- Chronic cavitary pulmonary aspergillosis, ABPA – Allergic bronchopulmonary aspergillosis, CNPA – Chronic necrotizing pulmonary aspergillosis, HSCT- Hematopoietic stem cell transplantation, EORTC- European Organization for Research and Treatment of Cancer.

Table 2.
Summary of immune system related genes mediating susceptibility to aspergillosis.

the association and impact of the complex interactions between the gut/pulmonary microbiome and the immune system in *Aspergillus*-related diseases. An understanding of the immune pathogenesis of aspergillosis can help in the development of strategies targeting *Aspergillus* itself as well as pulmonary or systemic immunity by influencing the host immune system, the microbiome and/or its metabolites.

Acknowledgements

All artworks are original and was prepared using the trial version of the online Biorender software.

Author details

Shreya Singh, Rimjhim Kanaujia and Shivaprakash M. Rudramurthy*
Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

*Address all correspondence to: mrshivprakash@yahoo.com

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Chakrabarti A: *Fungal infections in Asia : Eastern frontier of mycology*. Elsevier India 2014.
- [2] Chakrabarti A, Chatterjee SS, Das A, Shivaprakash MR: Invasive aspergillosis in developing countries. *Medical Mycology* 2011:S35–S47.
- [3] Holdom MD, Hay RJ, Hamilton AJ: Purification, n-terminal amino acid sequence and partial characterization of a Cu,Zn superoxide dismutase from the pathogenic fungus *Aspergillus fumigatus*. *Free Radic Res* 1995, 22:519-531.
- [4] Kothary MH, Chase T, Macmillan JD: Correlation of elastase production by some strains of *Aspergillus fumigatus* with ability to cause pulmonary invasive aspergillosis in mice. *Infect Immun* 1984, 43:320-325.
- [5] Ramesh M V, Sirakova T, Kolattukudy PE: Isolation, characterization, and cloning of cDNA and the gene for an elastinolytic serine proteinase from *Aspergillus flavus*. *Infect Immun* 1994, 62:79-85.
- [6] Sirakova TD, Markaryan A, Kolattukudy PE: Molecular cloning and sequencing of the cDNA and gene for a novel elastinolytic metalloproteinase from *Aspergillus fumigatus* and its expression in *Escherichia coli*. *Infect Immun* 1994, 62:4208-4218.
- [7] Reichard U, Eiffert H, Röchel R: Purification and characterization of an extracellular aspartic proteinase from *Aspergillus fumigatus*. *Med Mycol* 1994, 32:427-436.
- [8] Calera JA, Paris S, Monod M, Hamilton AJ, Debeaupuis JP, Diaquin M, López-Medrano R, Leal F, Latgé JP: Cloning and disruption of the antigenic catalase gene of *Aspergillus fumigatus*. *Infect Immun* 1997, 65:4718-4724.
- [9] Krappmann S, Bignell EM, Reichard U, Rogers T, Haynes K, Braus GH: The *Aspergillus fumigatus* transcriptional activator CpcA contributes significantly to the virulence of this fungal pathogen. *Mol Microbiol* 2004, 52:785-799.
- [10] Mullbacher A, Eichner RD: Immunosuppression in vitro by a metabolite of a human pathogenic fungus. *Proc Natl Acad Sci* 1984, 81:3835-3837.
- [11] Mullbacher A, Waring P, Eichner Rd: Identification of an agent in cultures of *Aspergillus fumigatus* displaying anti-phagocytic and immunomodulating activity in vitro. *Microbiology* 1985, 131:1251-1258.
- [12] Latgé JP, Moutaouakil M, Debeaupuis JP, Bouchara JP, Haynes K, Prévost MC: The 18-kilodalton antigen secreted by *Aspergillus fumigatus*. *Infect Immun* 1991, 59:2586-2594.
- [13] Paris S, Monod M, Diaquin M, Lamy B, Arruda LK, Punt PJ, Latgé JP: A transformant of *Aspergillus fumigatus* deficient in the antigenic cytotoxin ASPFI. *FEMS Microbiol Lett* 1993, 111:31-36.
- [14] Robens JF, Richard JL: Aflatoxins in Animal and Human Health. *Rev Environ Contam Toxicol*. 1992:69-94.
- [15] Eissenberg LG, Schlesinger PH, Goldman WE: Phagosome-Lysosome Fusion in P388D1 macrophages infected with *Histoplasma capsulatum*. *J Leukoc Biol* 1988, 43:483-491.
- [16] Hermanowski-Vosatka A, Detmers PA, Götze O, Silverstein SC, Wright SD: Clustering of ligand on the surface of a particle enhances adhesion to receptor-bearing cells. *J Biol Chem* 1988, 263:17822-7.

- [17] Valsecchi I, Dupres V, Stephen-Victor E, Guijarro JI, Gibbons J, Beau R, Bayry J, Coppee JY, Lafont F, et al.: Role of Hydrophobins in *Aspergillus fumigatus*. *J Fungi* (Basel). 2017, 24;4:2.
- [18] Alastruey-Izquierdo A, Cadranel J, Flick H, Godet C, Hennequin C, Hoenigl M, Kosmidis C, Lange C, Munteanu O, Page I, et al.: Treatment of Chronic Pulmonary Aspergillosis: Current Standards and Future Perspectives. *Respiration* 2018, 96:159-170.
- [19] Denning DW, Cadranel J, Beigelman-Aubry C, Ader F, Chakrabarti A, Blot S, Ullmann AJ, Dimopoulos G, Lange C: Chronic pulmonary aspergillosis: Rationale and clinical guidelines for diagnosis and management. *Eur Respir J* 2016, 47:45-68.
- [20] Agarwal R, Chakrabarti A, Shah A, Gupta D, Meis JF, Guleria R, Moss R, Denning DW: Allergic bronchopulmonary aspergillosis: Review of literature and proposal of new diagnostic and classification criteria. *Clin Exp Allergy* 2013, doi:10.1111/cea.12141.
- [21] Singh M, Paul N, Singh S, Nayak GR: Asthma and Fungus: Role in Allergic Bronchopulmonary Aspergillosis (ABPA) and Other Conditions. *Indian J Pediatr* 2018, 85:899-904.
- [22] Azie N, Neofytos D, Pfaller M, Meier-Kriesche HU, Quan SP, Horn D: The PATH (Prospective Antifungal Therapy) Alliance® registry and invasive fungal infections: Update 2012. *Diagn Microbiol Infect Dis* 2012, 73:293-300.
- [23] Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC: Hidden Killers: Human Fungal Infections. *Sci Transl Med* 2012, 4:165rv13-165rv13.
- [24] Alangaden GJ, Wahiduzzaman M, Chandrasekar PH: Aspergillosis: The Most Common Community-Acquired Pneumonia with Gram-Negative Bacilli as Copathogens in Stem Cell Transplant Recipients with Graft-versus-Host Disease. *Clin Infect Dis* 2002, 35:659-664.
- [25] Schauwvlieghe AFAD, Rijnders BJA, Philips N, Verwijs R, Vanderbeke L, Van Tienen C, Lagrou K, Verweij PE, Van de Veerdonk FL, Gommers D, et al.: Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. *Lancet Respir Med* 2018, 6:782-792.
- [26] Drummond RA, Franco LM, Lionakis MS: Human CARD9: A Critical Molecule of Fungal Immune Surveillance. *Front Immunol* 2018, 9.
- [27] Hodiament CJ, Dolman KM, Tenberge IJM, Melchers WJG, Verweij PE, Pajkrt D: Multiple-azole-resistant *Aspergillus fumigatus* osteomyelitis in a patient with chronic granulomatous disease successfully treated with long-term oral posaconazole and surgery. *Med Mycol* 2009, 47:217-220.
- [28] Mousavi B, Hedayati MT, Hedayati N, Ilkit M, Syedmousavi S: *Aspergillus* species in indoor environments and their possible occupational and public health hazards. *Curr Med Mycol* 2016, 2:36-42.
- [29] Hedayati MT, Mohseni-Bandpi A, Moradi S: A survey on the pathogenic fungi in soil samples of potted plants from Sari hospitals, Iran. *J Hosp Infect* 2004, 58:59-62.
- [30] Dagenais TRT, Keller NP: Pathogenesis of *Aspergillus fumigatus* in invasive aspergillosis. *Clin Microbiol Rev* 2009, 22:447-465.
- [31] Vogl G, Lesiak I, Jensen DB, Perkhofer S, Eck R, Speth C,

Lass-Flörl C, Zipfel PF, Blom AM, Dierich MP, et al.: Immune evasion by acquisition of complement inhibitors: The mould *Aspergillus* binds both factor H and C4b binding protein. *Mol Immunol* 2008, 45:1485-1493.

[32] Washburn RG, DeHart DJ, Agwu DE, Bryant-Varela BJ, Julian NC: *Aspergillus fumigatus* complement inhibitor: production, characterization, and purification by hydrophobic interaction and thin-layer chromatography. *Infect Immun* 1990, 58.

[33] Kogan TV, Jadoun J, Mittelman L, Hirschberg K, Osherov N: Involvement of Secreted *Aspergillus fumigatus* Proteases in Disruption of the Actin Fiber Cytoskeleton and Loss of Focal Adhesion Sites in Infected A549 Lung Pneumocytes. *J Infect Dis* 2004, 189:1965-1973.

[34] Arias M, Santiago L, Vidal-García M, Redrado S, Lanuza P, Comas L, Domingo MP, Rezusta A, Gálvez EM: Preparations for invasion: Modulation of host lung immunity during pulmonary aspergillosis by gliotoxin and other fungal secondary metabolites. *Front Immunol* 2018, 9:2549.

[35] Sales-Campos H, Tonani L, Cardoso CRB, Kress MRVZ: The immune interplay between the host and the pathogen in *Aspergillus fumigatus* lung infection. *Biomed Res Int* 2013, 2013.

[36] Rosentul DC, Delsing CE, Jaeger M, Plantinga TS, Oosting M, Costantini I, Venselaar H, Joosten LAB, van der Meer JWM, Dupont B, et al.: Gene polymorphisms in pattern recognition receptors and susceptibility to idiopathic recurrent vulvovaginal candidiasis. *Front Microbiol* 2014, 5:483.

[37] Netea MG, Warris A, Van Der Meer JWM, Fenton MJ, Verver-Janssen TJG, Jacobs LEH, Andresen T, Verweij PE, Kullberg BJ:

Aspergillus fumigatus evades immune recognition during germination through loss of toll-like receptor-4-mediated signal transduction. *J Infect Dis* 2003, 188:320-326.

[38] Bochud P-Y, Chien JW, Marr KA, Leisenring WM, Upton A, Janer M, Rodrigues SD, Li S, Hansen JA, Zhao LP, et al.: Toll-like Receptor 4 Polymorphisms and Aspergillosis in Stem-Cell Transplantation. *N Engl J Med* 2008, 359:1766-1777.

[39] Moreira AP, Cavassani KA, Ismailoglu UB, Hullinger R, Dunleavy MP, Knight DA, Kunkel SL, Uematsu S, Akira S, Hogaboam CM: The protective role of TLR6 in a mouse model of asthma is mediated by IL-23 and IL-17A. *J Clin Invest* 2011, 121:4420-4432.

[40] Bretz C, Gersuk G, Knoblauch S, Chaudhary N, Randolph-Habecker J, Hackman RC, Staab J, Marr KA: MyD88 signaling contributes to early pulmonary responses to *Aspergillus fumigatus*. *Infect Immun* 2008, 76:952-958.

[41] Bellocchio S, Montagnoli C, Bozza S, Gaziano R, Rossi G, Mambula SS, Vecchi A, Mantovani A, Levitz SM, Romani L: The Contribution of the Toll-Like/IL-1 Receptor Superfamily to Innate and Adaptive Immunity to Fungal Pathogens In Vivo. *J Immunol* 2004, 172:3059-3069.

[42] Dubourdeau M, Athman R, Balloy V, Huerre M, Chignard M, Philpott DJ, Latgé J-P, Ibrahim-Granet O: *Aspergillus fumigatus* Induces Innate Immune Responses in Alveolar Macrophages through the MAPK Pathway Independently of TLR2 and TLR4. *J Immunol* 2006, 177:3994-4001.

[43] Li ZZ, Tao LL, Zhang J, Zhang HJ, Qu JM: Role of NOD2 in regulating the immune response to *Aspergillus*

fumigatus. *Inflamm Res* 2012, 61: 643-648.

[44] Philippe B, Ibrahim-Granet O, Prévost MC, Gougerot-Pocidalo MA, Perez MS, Van der Meeren A, Latgé JP: Killing of *Aspergillus fumigatus* by alveolar macrophages is mediated by reactive oxidant intermediates. *Infect Immun* 2003, 71:3034-3042.

[45] De Castro CMMB, Manhães De Castro R, Fernandes De Medeiros A, Queirós Santos A, Ferreira E Silva WT, De Lima Filho JL: Effect of stress on the production of O₂- in alveolar macrophages. *J Neuroimmunol* 2000, 108:68-72.

[46] Reeves EP, Lu H, Jacobs HL, Messina CGM, Bolsover S, Gabellall G, Potma EO, Warley A, Roes J, Segal AW: Killing activity of neutrophils is mediated through activation of proteases by K⁺ flux. *Nature* 2002, 416:291-297.

[47] Ibrahim-Granet O, Philippe B, Boleti H, Boisvieux-Ulrich E, Grenet D, Stern M, Latgé JP: Phagocytosis and intracellular fate of *Aspergillus fumigatus* conidia in alveolar macrophages. *Infect Immun* 2003, 71:891-903.

[48] Lapp K, Vödisch M, Kroll K, Strassburger M, Kniemeyer O, Heinekamp T, Brakhage AA: Characterization of the *Aspergillus fumigatus* detoxification systems for reactive nitrogen intermediates and their impact on virulence. *Front Microbiol* 2014, 5:469.

[49] Hsieh SH, Kurzai O, Brock M: Persistence within dendritic cells marks an antifungal evasion and dissemination strategy of *Aspergillus terreus*. *Sci Rep* 2017, 7:1-11.

[50] Bhatia S, Fei M, Yarlagaadda M, Qi Z, Akira S, Saijo S, Iwakura Y, van Rooijen N, Gibson GA, St. Croix CM, et al.: Rapid host defense against

Aspergillus fumigatus involves alveolar macrophages with a predominance of alternatively activated phenotype. *PLoS One* 2011, 6.

[51] Braem SGE, Rooijackers SHM, van Kessel KPM, de Cock H, Wösten HAB, van Strijp JAG, Haas P-JA: Effective Neutrophil Phagocytosis of *Aspergillus fumigatus* Is Mediated by Classical Pathway Complement Activation. *J Innate Immun* 2015, 7:364-374.

[52] Snarr BD, St-Pierre G, Ralph B, Lehoux M, Sato Y, Rancourt A, Takazono T, Baistrocchi SR, Corsini R, Cheng MP, et al.: Galectin-3 enhances neutrophil motility and extravasation into the airways during *Aspergillus fumigatus* infection. *PLOS Pathog* 2020, 16:e1008741.

[53] Feldmesser M: Role of neutrophils in invasive aspergillosis. *Infect Immun* 2006, 74:6514-6516.

[54] Gazendam RP, van Hamme JL, Tool ATJ, Hoogenboezem M, van den Berg JM, Prins JM, Vitkov L, van de Veerdonk FL, van den Berg TK, Roos D, et al.: Human Neutrophils Use Different Mechanisms To Kill *Aspergillus fumigatus* Conidia and Hyphae: Evidence from Phagocyte Defects. *J Immunol* 2016, 196:1272-1283.

[55] Leal SM, Roy S, Vareechon C, Carrion S de J, Clark H, Lopez-Berges MS, diPietro A, Schrettl M, Beckmann N, Redl B, et al.: Targeting Iron Acquisition Blocks Infection with the Fungal Pathogens *Aspergillus fumigatus* and *Fusarium oxysporum*. *PLoS Pathog* 2013, 9.

[56] Clark HL, Jhingran A, Sun Y, Vareechon C, de Jesus Carrion S, Skaar EP, Chazin WJ, Calera JA, Hohl TM, Pearlman E: Zinc and Manganese Chelation by Neutrophil S100A8/A9 (Calprotectin) Limits Extracellular *Aspergillus fumigatus*

Hyphal Growth and Corneal Infection .
J Immunol 2016, 196:336-344.

against *Aspergillus fumigatus*. *Blood*
2006, 107:2562-2569.

[57] McCormick A, Heesemann L, Wagener J, Marcos V, Hartl D, Loeffler J, Heesemann J, Ebel F: NETs formed by human neutrophils inhibit growth of the pathogenic mold *Aspergillus fumigatus*. *Microbes Infect* 2010, 12:928-936.

[64] Vogel K, Pierau M, Arra A, Lampe K, Schlueter D, Arens C, Brunner-Weinzierl MC: Developmental induction of human T-cell responses against *Candida albicans* and *Aspergillus fumigatus*. *Sci Rep* 2018, 8:16904.

[58] Park SJ, Hughes MA, Burdick M, Strieter RM, Mehrad B: Early NK Cell-Derived IFN- γ Is Essential to Host Defense in Neutropenic Invasive Aspergillosis. *J Immunol* 2009, 182:4306-4312.

[65] Jolink H, de Boer R, Hombrink P, Jonkers RE, van Dissel JT, Falkenburg JHF, Heemskerk MHM: Pulmonary immune responses against *Aspergillus fumigatus* are characterized by high frequencies of IL-17 producing T-cells. *J Infect* 2017, 74:81-88.

[59] Santiago V, Rezvani K, Sekine T, Stebbing J, Kelleher P, Armstrong-James D: Human NK Cells Develop an Exhaustion Phenotype During Polar Degranulation at the *Aspergillus fumigatus* Hyphal Synapse. *Front Immunol* 2018, 9:2344.

[66] Lilly LM, Gessner MA, Dunaway CW, Metz AE, Schwiebert L, Weaver CT, Brown GD, Steele C: The β -Glucan Receptor Dectin-1 Promotes Lung Immunopathology during Fungal Allergy via IL-22. *J Immunol* 2012, 189:3653-3660.

[60] Zhang X, He D, Gao S, Wei Y, Wang L: *Aspergillus fumigatus* enhances human NK cell activity by regulating M1 macrophage polarization. *Mol Med Rep* 2019, 20:1241-1249.

[67] Murdock BJ, Shreiner AB, McDonald RA, Osterholzer JJ, White ES, Toews GB, Huffnagle GB: Coevolution of TH1, TH2, and TH17 responses during repeated pulmonary exposure to *Aspergillus fumigatus* conidia. *Infect Immun* 2011, 79:125-135.

[61] Reizis B, Bunin A, Ghosh HS, Lewis KL, Sisirak V: Plasmacytoid dendritic cells: Recent progress and open questions. *Annu Rev Immunol* 2011, 29:163-183.

[68] Shreiner AB, Murdock BJ, Akha AAS, Falkowski NR, Christensen PJ, White ES, Hogaboam CM, Huffnagle GB: Repeated exposure to *Aspergillus fumigatus* conidia results in CD4 + T cell-dependent and -independent pulmonary arterial remodeling in a mixed th1/th2/th17 microenvironment that requires interleukin-4 (IL-4) and IL-10. *Infect Immun* 2012, 80:388-397.

[62] Bozza S, Gaziano R, Spreca A, Bacci A, Montagnoli C, di Francesco P, Romani L: Dendritic Cells Transport Conidia and Hyphae of *Aspergillus fumigatus* from the Airways to the Draining Lymph Nodes and Initiate Disparate Th Responses to the Fungus. *J Immunol* 2002, 168:1362-1371.

[69] Arora S, Olszewski MA, Tsang TM, McDonald RA, Toews GB, Huffnagle GB: Effect of cytokine interplay on macrophage polarization during chronic pulmonary infection with *Cryptococcus neoformans*. *Infect Immun* 2011, 79:1915-1926.

[63] Beck O, Topp MS, Koehl U, Roilides E, Simitsopoulou M, Hanisch M, Sarfati J, Latgé JP, Klingebiel T, Einsele H, et al.: Generation of highly purified and functionally active human TH1 cells

- [70] Shiang C, Mauad T, Senhorini A, De Araújo BB, Ferreira DS, Da Silva LFF, Dolhnikoff M, Tsokos M, Rabe KF, Pabst R: Pulmonary periarterial inflammation in fatal asthma. *Clin Exp Allergy* 2009, 39:1499-1507.
- [71] Dewi IMW, van de Veerdonk FL, Gresnigt MS: The multifaceted role of T-helper responses in host defense against *Aspergillus fumigatus*. *J Fungi* 2017, 3.
- [72] Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, Mellor AL: GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity* 2005, 22:633-642.
- [73] Iannitti RG, Carvalho A, Cunha C, De Luca A, Giovannini G, Casagrande A, Zelante T, Vacca C, Fallarino F, Puccetti P, et al.: Th17/Treg imbalance in murine cystic fibrosis is linked to indoleamine 2,3-dioxygenase deficiency but corrected by kynurenines. *Am J Respir Crit Care Med* 2013, 187:609-620.
- [74] Montagnoli C, Bozza S, Bacci A, Gaziano R, Mosci P, Morschhäuser J, Pitzurra L, Kopf M, Cutler J, Romani L: A role for antibodies in the generation of memory antifungal immunity. *Eur J Immunol* 2003, 33:1193-1204.
- [75] Chen K, Xu W, Wilson M, He B, Miller NW, Bengtén E, Edholm ES, Santini PA, Rath P, Chiu A, et al.: Immunoglobulin D enhances immune surveillance by activating antimicrobial, proinflammatory and B cell-stimulating programs in basophils. *Nat Immunol* 2009, 10:889-898.
- [76] Boita Enrico Heffler Stefano Pizzimenti Alberto Raie Elona Saraci Paola Omedè Claudia Bussolino Caterina Bucca Giovanni Rolla M, Mauriziano Umberto OI: Regulation of B-Cell-Activating Factor Expression on the Basophil Membrane of Allergic Patients. *Int Arch Allergy Immunol* 2015, 166:208-212.
- [77] Kim CH: Immune regulation by microbiome metabolites. *Immunology* 2018, 154:220-229.
- [78] Galloway-Peña JR, Kontoyiannis DP: The gut mycobiome: The overlooked constituent of clinical outcomes and treatment complications in patients with cancer and other immunosuppressive conditions. *PLOS Pathog* 2020, 16:e1008353.
- [79] Wu T, Li H, Su C, Xu F, Yang G, Sun K, Xu M, Lv N, Meng B, Liu Y, et al.: Microbiota-Derived Short-Chain Fatty Acids Promote LAMTOR2-Mediated Immune Responses in Macrophages. *mSystems* 2020, 5.
- [80] Schulthess J, Pandey S, Capitani M, Rue-Albrecht KC, Arnold I, Franchini F, Chomka A, Ilott NE, Johnston DGW, Pires E, et al.: The Short Chain Fatty Acid Butyrate Imprints an Antimicrobial Program in Macrophages. *Immunity* 2019, 50:432-445.e7.
- [81] Ciarlo E, Heinonen T, Herderschee J, Fenwick C, Mombelli M, Le Roy D, Roger T: Impact of the microbial derived short chain fatty acid propionate on host susceptibility to bacterial and fungal infections in vivo. *Sci Rep* 2016, 6:1-15.
- [82] Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, Blanchard C, Junt T, Nicod LP, Harris NL, et al.: Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* 2014, 20:159-166.
- [83] Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch S V., et al.: Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell* 2009, 139:485-498.

- [84] McAleer JP, Nguyen NLH, Chen K, Kumar P, Ricks DM, Binnie M, Armentrout RA, Pociask DA, Hein A, Yu A, et al.: Pulmonary Th17 antifungal immunity is regulated by the gut microbiome. *J Immunol* 2016, 197:97-107.
- [85] Ganai SC, Sanos SL, Kallfass C, Oberle K, Johner C, Kirschning C, Lienenklaus S, Weiss S, Staeheli P, Aichele P, et al.: Priming of natural killer cells by nonmucosal mononuclear phagocytes requires instructive signals from commensal microbiota. *Immunity* 2012, 37:171-186.
- [86] Abt MC, Osborne LC, Monticelli LA, Doering TA, Alenghat T, Sonnenberg GF, Paley MA, Antenus M, Williams KL, Erikson J, et al.: Commensal bacteria calibrate the activation threshold of innate antiviral immunity. *Immunity* 2012, 37: 158-170.
- [87] Sonnenberg GF, Artis D: Innate Lymphoid Cell Interactions with Microbiota: Implications for Intestinal Health and Disease. *Immunity* 2012, 37:601-610.
- [88] Crellin NK, Trifari S, Kaplan CD, Satoh-Takayama N, Di Santo JP, Spits H: Regulation of cytokine secretion in human CD127⁺ LTi-like innate lymphoid cells by toll-like receptor 2. *Immunity* 2010, 33:752-764.
- [89] Hill DA, Siracusa MC, Abt MC, Kim BS, Kobuley D, Kubo M, Kambayashi T, Larosa DF, Renner ED, Orange JS, et al.: Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat Med* 2012, 18:538-546.
- [90] Pragman AA, Kim HB, Reilly CS, Wendt C, Isaacson RE: The Lung Microbiome in Moderate and Severe Chronic Obstructive Pulmonary Disease. *PLoS One* 2012, 7.
- [91] Lynch S V.: Viruses and microbiome alterations. In *Annals of the American Thoracic Society*. . Ann Am Thorac Soc; 2014.
- [92] Leung RKK, Zhou JW, Guan W, Li SK, Yang ZF, Tsui SKW: Modulation of potential respiratory pathogens by pH1N1 viral infection. *Clin Microbiol Infect* 2013, 19:930-935.
- [93] Noverr MC, Noggle RM, Toews GB, Huffnagle GB: Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. *Infect Immun* 2004, 72:4996-5003.
- [94] Amin R, Dupuis A, Aaron SD, Ratjen F: The effect of chronic infection with *Aspergillus fumigatus* on lung function and hospitalization in patients with cystic fibrosis. *Chest* 2010, 137:171-176.
- [95] Baxter CG, Rautemaa R, Jones AM, Kevin Webb A, Bull M, Mahenthiralingam E, Denning DW: Intravenous antibiotics reduce the presence of *Aspergillus* in adult cystic fibrosis sputum. *Thorax* 2013, 68:652-657.
- [96] Kesh S, Mensah NY, Peterlongo P, Jaffe D, Hsu K, VAN DEN Brink M, O'reilly R, Pamer E, Satagopan J, Papanicolaou GA: TLR1 and TLR6 polymorphisms are associated with susceptibility to invasive aspergillosis after allogeneic stem cell transplantation. *Ann N Y Acad Sci* 2005, 1062:95-103.
- [97] Lamoth F, Rubino I, Bochud P-Y: Immunogenetics of invasive aspergillosis. *Med Mycol* 2011, 49:S125-S136.
- [98] Carvalho A, Pasqualotto AC, Pitzurra L, Romani L, Denning DW, Rodrigues F: Polymorphisms in toll-like receptor genes and susceptibility to pulmonary aspergillosis. *J Infect Dis* 2008, 197:618-621.

- [99] de Boer MGJ, Jolink H, Halkes CJM, van der Heiden PLJ, Kremer D, Falkenburg JHF, van de Vosse E, van Dissel JT: Influence of Polymorphisms in Innate Immunity Genes on Susceptibility to Invasive Aspergillosis after Stem Cell Transplantation. *PLoS One* 2011, 6:e18403.
- [100] Grube M, Loeffler J, Mezger M, Krüger B, Echtenacher B, Hoffmann P, Edinger M, Einsele H, Andreesen R, Holler E: TLR5 stop codon polymorphism is associated with invasive aspergillosis after allogeneic stem cell transplantation. *Med Mycol* 2013, 51:818-825.
- [101] Pamer EG: TLR Polymorphisms and the Risk of Invasive Fungal Infections. *N Engl J Med* 2008, 359:1836-1838.
- [102] Cunha C, Di Ianni M, Bozza S, Giovannini G, Zagarella S, Zelante T, D'Angelo C, Pierini A, Pitzurra L, Falzetti F, et al.: Dectin-1 Y238X polymorphism associates with susceptibility to invasive aspergillosis in hematopoietic transplantation through impairment of both recipient- and donor-dependent mechanisms of antifungal immunity. *Blood* 2010, 116:5394-5402.
- [103] Chai LYA, de Boer MGJ, van der Velden WJFM, Plantinga TS, van Spruel AB, Jacobs C, Halkes CJM, Vonk AG, Blijlevens NM, van Dissel JT, et al.: The Y238X Stop Codon Polymorphism in the Human β -Glucan Receptor Dectin-1 and Susceptibility to Invasive Aspergillosis. *J Infect Dis* 2011, 203:736-743.
- [104] Sainz J, Lupiáñez CB, Segura-Catena J, Vazquez L, Ríos R, Oyonarte S, Hemminki K, Försti A, Jurado M: Dectin-1 and DC-SIGN polymorphisms associated with invasive pulmonary aspergillosis infection. *PLoS One* 2012, 7.
- [105] Cunha C, Aversa F, Lacerda JF, Busca A, Kurzai O, Grube M, Löffler J, Maertens JA, Bell AS, Inforzato A, et al.: Genetic PTX3 Deficiency and Aspergillosis in Stem-Cell Transplantation. *N Engl J Med* 2014, 370:421-432.
- [106] Wójtowicz A, Lecompte TD, Bibert S, Manuel O, Rüeger S, Berger C, Boggian K, Cusini A, Garzoni C, Hirsch H, et al.: PTX3 Polymorphisms and Invasive Mold Infections after Solid Organ Transplant. *Clin Infect Dis* 2015, 61:619-622.
- [107] Sainz J, Pérez E, Hassan L, Moratalla A, Romero A, Collado MD, Jurado M: Variable Number of Tandem Repeats of TNF Receptor Type 2 Promoter as Genetic Biomarker of Susceptibility to Develop Invasive Pulmonary Aspergillosis. *Hum Immunol* 2007, 68:41-50.
- [108] Vaid M, Kaur S, Sambatakou H, Madan T, Denning DW, Sarma PU: Distinct alleles of mannose-binding lectin (MBL) and surfactant proteins A (SP-A) in patients with chronic cavitary pulmonary aspergillosis and allergic bronchopulmonary aspergillosis. *Clin Chem Lab Med* 2007, 45:183-186.
- [109] Kaur S, Gupta VK, Thiel S, Sarma PU, Madan T: Protective role of mannan-binding lectin in a murine model of invasive pulmonary aspergillosis. *Clin Exp Immunol* 2007, 148:382-389.
- [110] Crosdale DJ, Poulton K V, Ollier WE, Thomson W, Denning DW: Mannose-binding lectin gene polymorphisms as a susceptibility factor for chronic necrotizing pulmonary aspergillosis. *J Infect Dis* 2001, 184:653-656.
- [111] Borta S, Popetiu R, Donath-Miklos I, Puschita M: Genetic Polymorphism of MBL 2 in Patients

with Allergic Bronchial Asthma.
Maedica (Buchar) 2019, 14:208-212.

[112] Lambourne J, Agranoff D, Herbrecht R, Buchbinder A, Willis F, Letscher-Bru V, Agrawal S, Doffman S, Johnson E, White PL, et al.: Association of mannose-binding lectin deficiency with acute invasive aspergillosis in immunocompromised patients. *Clin Infect Dis* 2009, 49:1486-1491.

[113] Carvalho A, Cunha C, Di Ianni M, Pitzurra L, Aloisi T, Falzetti F, Carotti A, Bistoni F, Aversa F, Romani L: Prognostic significance of genetic variants in the IL-23/Th17 pathway for the outcome of T cell-depleted allogeneic stem cell transplantation. *Bone Marrow Transplant* 2010, 45:1645-1652.

[114] Zaas AK, Liao G, Chien JW, Weinberg C, Shore D, Giles SS, Marr KA, Usuka J, Burch LH, Perera L, et al.: Plasminogen alleles influence susceptibility to invasive aspergillosis. *PLoS Genet* 2008, 4.

[115] Cunha C, Rodrigues F, Zelante T, Aversa F, Romani L, Carvalho A: Genetic susceptibility to aspergillosis in allogeneic stem-cell transplantation. In *Medical Mycology*. . Oxford Academic; 2011:S137–S143.

[116] Tanpaibule T, Jinawath N, Taweewongsounton A, Niparuck P, Rotjanapan P: Genetic Risk Surveillance for Invasive Aspergillosis in Hematology Patients: A Prospective Observational Study. *Infect Dis Ther* 2020, 9:807-821.

[117] Saxena S, Madan T, Shah A, Muralidhar K, Sarma PU: Association of polymorphisms in the collagen region of SP-A2 with increased levels of total IgE antibodies and eosinophilia in patients with allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 2003, 111:1001-1007.

[118] Madan T, Kaur S, Saxena S, Singh M, Kishore U, Thiel S, Reid KBM, Sarma PU: Role of collectins in innate immunity against aspergillosis. *Med Mycol* 2005, 43:155-163.

[119] Guo Y, Kasahara S, Jhingran A, Tosini NL, Zhai B, Aufiero MA, Mills KAM, Gjonbalaj M, Espinosa V, Rivera A, et al.: During *Aspergillus* Infection, Monocyte-Derived DCs, Neutrophils, and Plasmacytoid DCs Enhance Innate Immune Defense through CXCR3-Dependent Crosstalk. *Cell Host Microbe* 2020, 28:104-116.e4.

[120] Fisher CE, Hohl TM, Fan W, Storer BE, Levine DM, Zhao LP, Martin PJ, Warren EH, Boeckh M, Hansen JA: Validation of single nucleotide polymorphisms in invasive aspergillosis following hematopoietic cell transplantation. *Blood* 2017, 129:2693-2701.

[121] Mezger M, Steffens M, Beyer M, Manger C, Eberle J, Toliat MR, Wienker TF, Ljungman P, Hebart H, Dornbusch HJ, et al.: Polymorphisms in the chemokine (C-X-C motif) ligand 10 are associated with invasive aspergillosis after allogeneic stem-cell transplantation and influence CXCL10 expression in monocyte-derived dendritic cells. *Blood* 2008, 111:534-536.

[122] Lupiañez CB, Martínez-Bueno M, Sánchez-Maldonado JM, Badiola J, Cunha C, Springer J, Lackner M, Segura-Catena J, Canet LM, Alcazar-Fuoli L, et al.: Polymorphisms within the ARNT2 and CX3CR1 genes are associated with the risk of developing invasive aspergillosis. *Infect Immun* 2020, 88:882-901.

[123] Lupiañez CB, Canet LM, Carvalho A, Alcazar-Fuoli L, Springer J, Lackner M, Segura-Catena J, Comino A, Olmedo C, Ríos R, et al.: Polymorphisms in host immunity-modulating genes and risk of invasive aspergillosis: Results

from the AspBIOmics Consortium.
Infect Immun 2016, 84:643-657.

[124] Brouard J, Knauer N, Boelle PY, Corvol H, Henrion-Caude A, Flamant C, Bremont F, Delaisi B, Duhamel JF, Marguet C, et al.: Influence of interleukin-10 on *Aspergillus fumigatus* infection in patients with cystic fibrosis. *J Infect Dis* 2005, 191:1988-1991.

[125] Seo KW, Kim DH, Sohn SK, Lee NY, Chang HH, Kim SW, Jeon SB, Baek JH, Kim JG, Suh JS, et al.: Protective role of interleukin-10 promoter gene polymorphism in the pathogenesis of invasive pulmonary aspergillosis after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2005, 36:1089-1095.

[126] Sainz J, Hassan L, Perez E, Romero A, Moratalla A, López-Fernández E, Oyonarte S, Jurado M: Interleukin-10 promoter polymorphism as risk factor to develop invasive pulmonary aspergillosis. *Immunol Lett* 2007, 109:76-82.

[127] Knutsen AP, Kariuki B, Consolino JD, Warriar MR: IL-4 alpha chain receptor (IL-4R α) polymorphisms in allergic bronchopulmonary aspergillosis. *Clin Mol Allergy* 2006, 4.

[128] Lupiáñez CB, Villaescusa MT, Carvalho A, Springer J, Lackner M, Sánchez-Maldonado JM, Canet LM, Cunha C, Segura-Catena J, Alcazar-Fuoli L, et al.: Common genetic polymorphisms within NF κ B-related genes and the risk of developing invasive aspergillosis. *Front Microbiol* 2016, 7:1243.