Chapter

Immunopathogenesis of Aspergillosis

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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 2. Metalloproteinase 3. Aspartic (acid) proteinase	Degrades elastin. Degrades fibrinogen and laminin. Assist in host cell invasion of the hyphae.	36-kDa 23-kDa	[4] [5] [6, 7]
Catalase	ROS scavengers. Breakdown hydrogen peroxide (H_2O_2) to oxygen and water.	*catA* - conidium-specific gene *cat2* - mycelium-specific gene	[8, 9]
Toxins			
1. Gliotoxin 2. Restrictocin 3. Aflatoxin	Inhibits macrophage phagocytosis. Induces fragmentation and apoptosis of DNA in macrophages. Inhibition of T-cell activation. 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 *in vitro*. Also, epidemiologically to hepatocellular carcinoma.	18-kDa cytotoxin gene cluster of aflatoxin biosynthesis regulated by *AflCDC14*	[10, 11] [12, 13] [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 *Aspergillus* 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 are 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 susceptibly to IA is also seen in patients

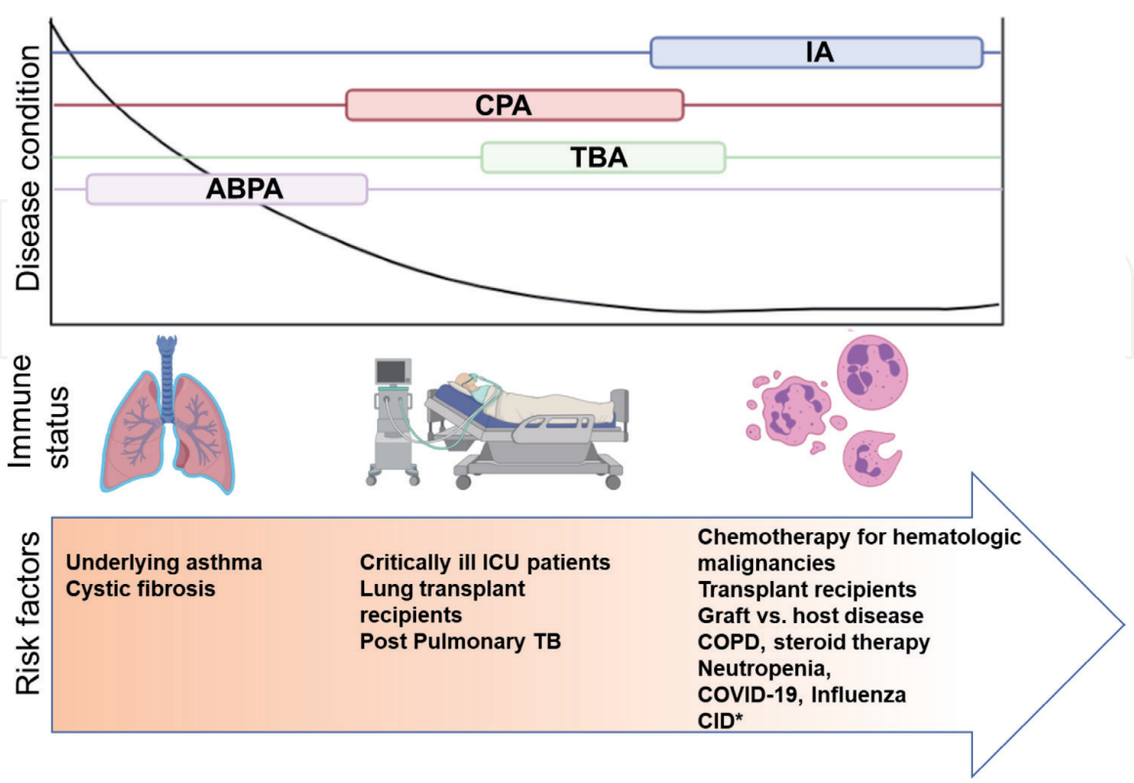

Figure 1.
Correction of clinical spectrum of Aspergillosis *and immune status in various condition. *CID: Congenital immunodeficiency disorders includes chronic granulomatous disease, CARD9 deficiency, leukocyte adhesion deficiency, Job's syndrome, pulmonary alveolar proteinosis.*

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

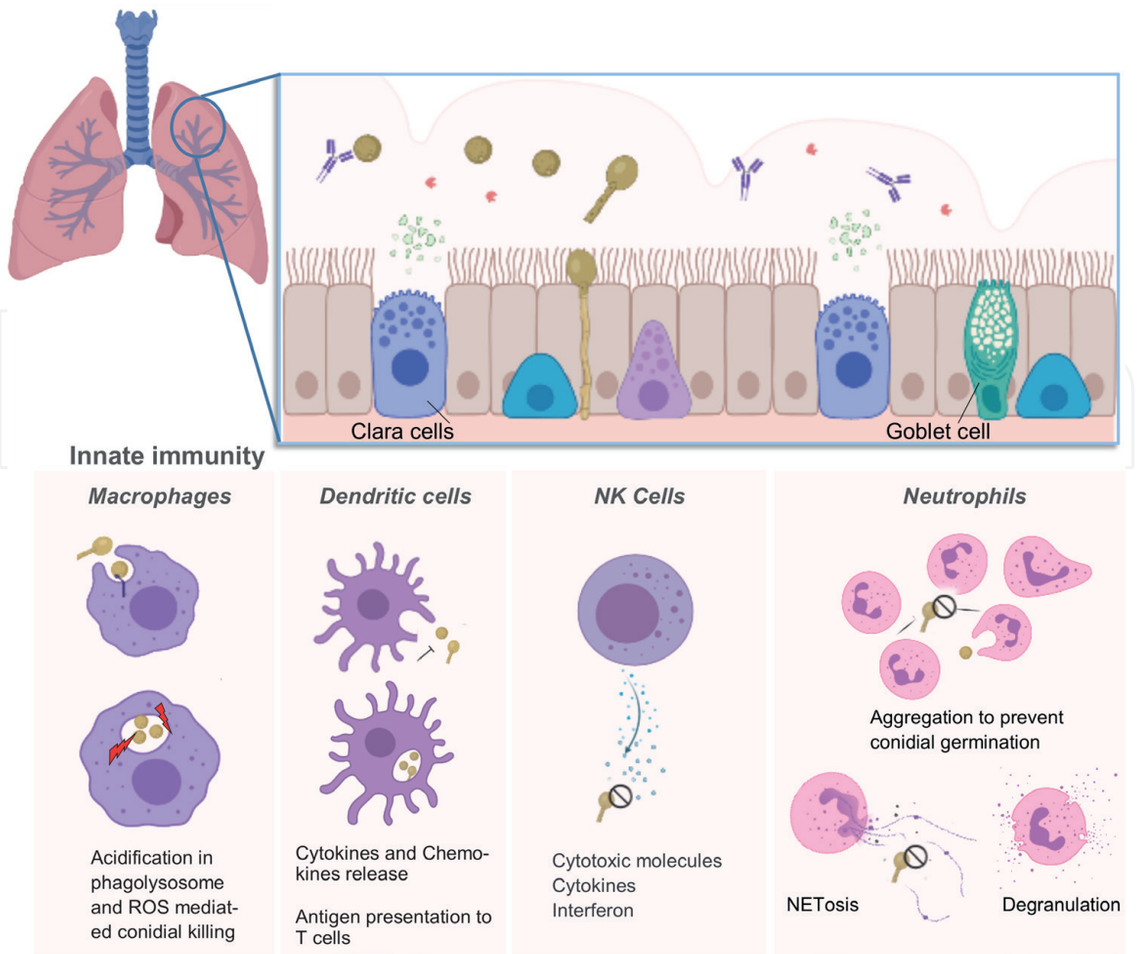

Figure 2.
Innate immune response to Aspergillus *infection. The conidia of* Aspergillus *spp. are inhaled and enter the lung where they encounter various soluble lung components including antibodies, complement factors and antimicrobial compounds. Those conidia which swell and undergoes germination further 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 to 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 mucocilliary 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*-nitrosoglutathione reductase (*GnoA*) which reduce *S*-nitrosoglutathione 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, dihydroxynapthalene-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 surprize 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 IFNα (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 FcγR 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

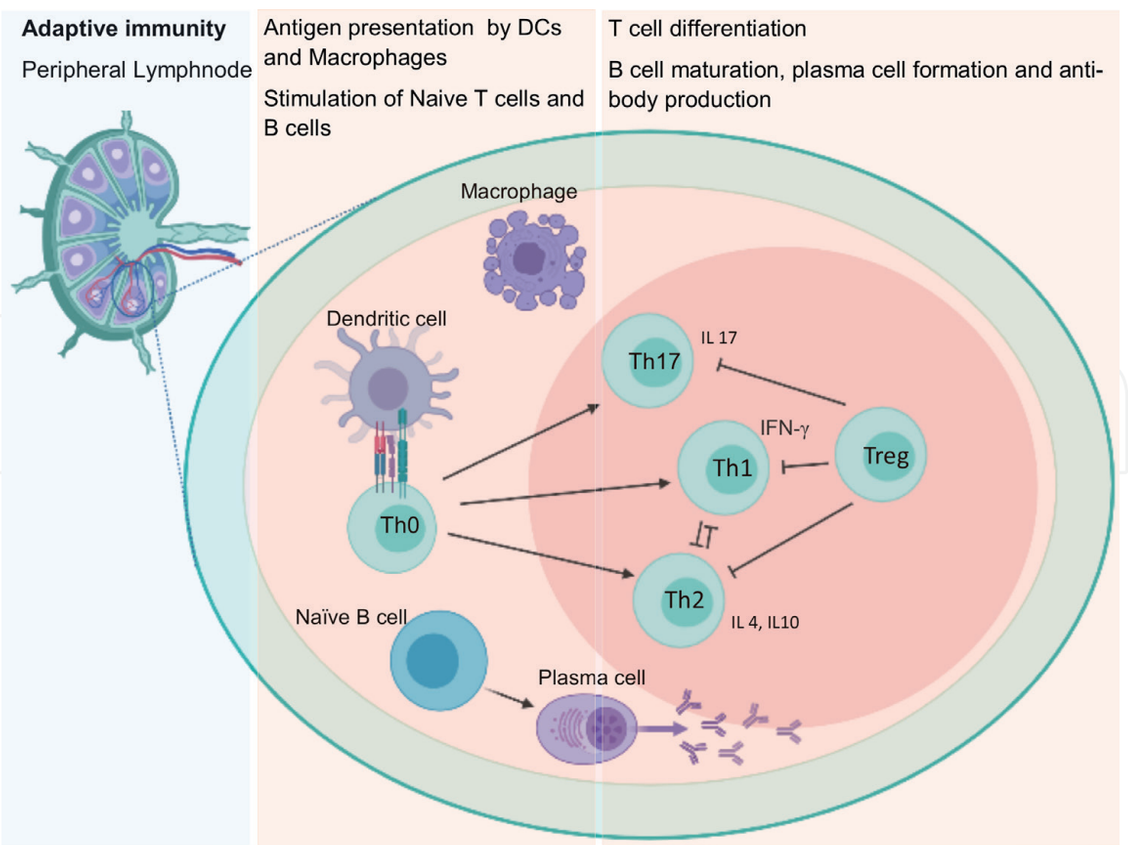

Figure 3.
Adaptive immune response to aspergillus *infection.* Aspergillus *spp. antigens are presented to naive T cells in peripheral lymphoid organs by dendritic cells and macrophages which further induces inflammation with coevolution of Th1, Th2, and Th17 response. B cells are also stimulated resulting 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 (μMT) 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 μMT 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 plaminogen) cytokines (*IL1, IL10,* IFN- γ, *CXCL10, ARNT2,)* and their receptors (*CX3CR1* and IL-4Rα) 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 *Aspergillus* 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]
Dectin-1	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]
Dectin-2	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]
DC-SIGN	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 *Aspergillus fumigatus* 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-ɤ	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 *Aspergillus fumigatus.*	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.

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