Chapter 4
When *Aspergillus fumigatus* Meets the Man

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Abstract *Aspergillus fumigatus* is one of the most ubiquitous opportunistic fungal pathogen, which can cause life-threatening invasive pulmonary infections in immunocompromised populations. Upon the inhalation of the *A. fumigatus* conidia, the encounter between the fungus and the host presents a complex interplay. This chapter will summarize the host innate immunity against *A. fumigatus*, and emphasize on the host immune evasion mechanisms of *A. fumigatus*.

4.1 Introduction

Aspergillus species are saprotrophic thermophilic fungi living in decaying material in the soil. When the fungus is starved, it produces millions of aerial conidia which are responsible for the propagation of the fungus (Fig. 4.1). Some of the *Aspergillus* species cause clinical manifestations ranging from chronic to invasive pulmonary infections, following the inhalation of the conidia (hundreds per day in normal environments) [1, 2]. *Aspergillus fumigatus* is the most prevalent etiologic agent of aspergillosis, followed by *Aspergillus flavus*, *A. niger*, *A. nidulans*, *A. terreus* [3]. In immunocompetent individuals, the inhaled conidia rarely cause infections since the host innate immunity is efficient in the clearance of the fungal pathogen [4–9] (Fig. 4.2). In the populations with pre-existing pulmonary cavities who are otherwise immunocompetent, colonization of *Aspergillus* species will only lead to chronic pulmonary aspergillosis (CPA) [10–12]. Unlike CPA, invasive pulmonary aspergillosis (IPA) predominantly affects immunocompromised individuals [13]. Individuals with primary or secondary immunodeficiency, such as chronic granulomatous disease, hematologic malignancy, hematopoietic stem cells transplantation, solid organ transplantation, and neutropenia consecutive to cancer chemotherapy or immunosuppressive treatment are typically at risk for IPA (Fig. 4.2) [2, 14]. The mortality rates of IPA vary among groups of host with different underlying risk factors, but it is generally high [2, 14]. *A. fumigatus* benefits from immune deficiencies, and it also possesses various well-established and specific strategies which helps the

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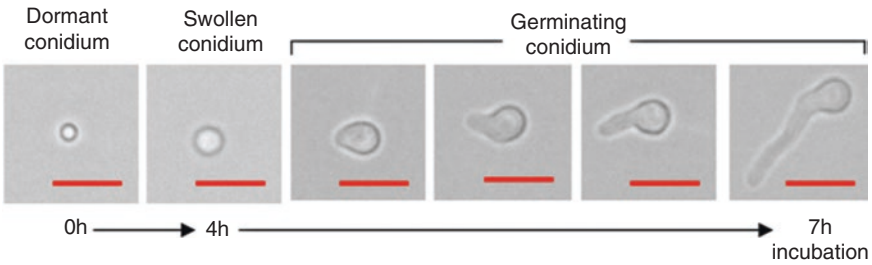

Fig. 4.1 Light microscopy (40×, scale bar = 10 μm) of the morphological change of *A. fumigatus* under incubation at 37 °C in Sabouraud medium

fungus to evade from the host debilitated immune system and colonize the lung parenchyma [15–20].

This chapter will dissect both the host innate immunity and the pathogen anti-immune strategies on the early stages of the fungal infection. Since contact between membrane-bound and soluble pattern recognition receptors (PRRs) of the host and surface pathogen-associated molecular patterns of *A. fumigatus* is the first event leading to the reciprocal recognition of the host and the pathogen (Fig. 4.3). It will especially focus on the host and pathogen molecules favoring their respective recognition, as well as the role of the innate immune cells involved in the anti-*A. fumigatus* defense. A thorough understanding of this complex "tug of war" between the host and the pathogen is fundamental in providing new insights in developing prophylactic strategies of IPA.

4.2 Molecules Responsible for the "Hide-and-Seek" Between Host and *Aspergillus fumigatus*

The fungal cell is surrounded by a cell wall with a specific composition which is very different from the phospholipid bilayer of the host cell plasma membrane. The *A. fumigatus* cell wall which surrounds the fungal cell is mainly composed of polysaccharides which are interlinked alkali insoluble β-1,3-glucans, chitin, and galactomannan and, alkali soluble α-1,3-glucans [21, 22]. These fungal structural polysaccharides are absent in mammalian cells and are therefore pathogen-associated molecular patterns (PAMPs), which are recognized by various membrane-bound and soluble PRRs of the host as foreign objects [21, 23–26].

In resting conidia, this polysaccharide core which is of similar composition both in the conidium and hyphal cell wall, is covered by a bilayered outer layer composed of hydrophobins (the most external) and melanin. The external hydrophobin rodlet layer, which is responsible for the hydrophobicity of the conidia is exclusively composed of the amyloid hydrophobic RodA proteins [27–29]. One way for *A. fumigatus* to become a pathogen is its capacity to "hide" from the host defensive

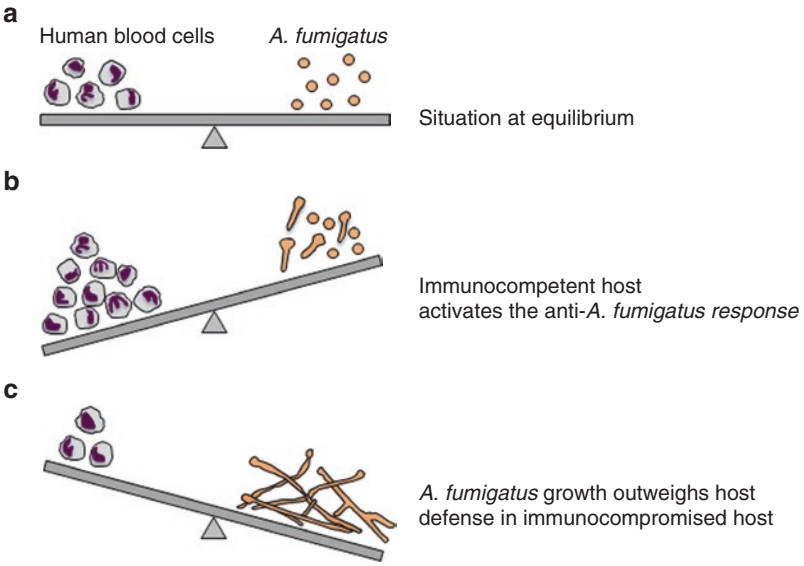

Fig. 4.2 Immune system–*Aspergillus fumigatus* interplay. The fungal disease is only established in the immunodeficient host, where the fungus can resist and escape the immune surveillance and establish the infection

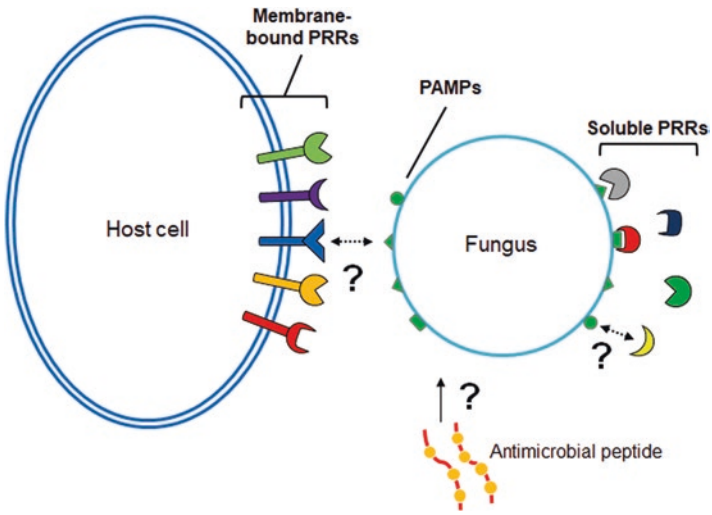

Fig. 4.3 Interaction between various membrane-bound and soluble PRRs which have been shown to bind to the PAMPs on the conidial surface. However, the corresponding PAMPs for each of the PRRs have not been elucidated (as indicated with *question mark*)

response immediately after inhalation and to go undetected after entering the respiratory tract [30–32]. This is due to the presence of the rodlet and melanin outer layers of the dormant conidia which hide the immunogenic cell wall polysaccharides. By doing so, an immediate strong inflammatory response, which would be detrimental for the fungus, is avoided. Moreover, by delaying the immune response, the initial survival of the fungus is prolonged in the host.

The loss of the rodlet and melanin layer during germination leads to an entire modification of the surface layer, which leads to the apparition of immunogenic polysaccharides on the hyphal surface. These surface molecules are recognized by PRRs that are mostly lectin receptors (carbohydrate-recognizing) involved in the initiation of the antifungal response. Indeed, conidial germination can be considered by the fungus as a form suicide. However, instead of being covered by melanin and RodA protein, the cell wall of the mycelium is covered by a specific hyphal galactosaminogalactan, which is immunosuppressive and favors the vegetative fungal growth. All these strategies for the host to seek for the fungal alien and for the fungus to counteract the host immune response after the immediate contact will be discussed below.

4.2.1 Membrane-Bound Pattern Recognition Receptors

4.2.1.1 Toll-Like Receptors

Toll-like receptors (TLRs) are a family of membrane-bound and soluble receptors on sentinel cells involved in the recognition of specific PAMPs [33]. Although the role of TLR2 and TLR4 in the immune defense against fungal pathogens have been extensively studied in the past decades [34]; so far, their precise functions have not yet been clearly elucidated. Various in vitro studies have demonstrated that TLR2 is involved in recognizing *A. fumigatus* [35, 36]. Blocking TLR2 led to a reduced phagocytic rate of *A. fumigatus* conidia, but not of control beads, suggesting that the phagocytic machinery is not impaired by TLR2 blocking [37]. Neutrophil-depleted TLR2-deficient mice had however a lower survival and produced less TNF-α upon stimulation with *A. fumigatus* [38]. The recruitment of neutrophils was severely attenuated in non-immunosuppressed mice deficient in both TLR2 and TLR4, when compared to that in mice with single deficiency [39]. Furthermore, the production of different cytokines in response to *A. fumigatus* is individually mediated by TLR2 and TLR4. For instance, the production of IL-12 and IL-6 from monocytes-derived dendritic cells are mediated by TLR2 and TLR4 respectively [40]. TLR4-mediated pro-inflammatory signals were lost during phenotypic switch from conidia to hyphae, contributing to the escape of the pathogens from the host immune defense [36, 41]. The presence of an optimal innate immune response required both TLR2 and TLR4. Although a direct binding has not been shown, polysaccharides of the cell wall seem to be recognized by TLR2 and TLR4. The IL-6 production via TLR2 and TLR4

stimulation in PBMCs (incubated with TLR2 and TLR4 ligands respectively) was attenuated by pre-incubation with α-glucan and galactomannan, while pre-incubation with β-glucan attenuated the IL-6 production via only TLR4 stimulation [42]. However, TLR2- and TLR4-knockout immunocompetent mice were not more susceptible to challenge of invasive aspergillosis, suggesting that these two receptors are not essential in preventing *Aspergillus* infection in an immunocompetent status [43].

Unlike TLR2 and TLR4, TLR9 is located intracellularly in the endosome compartment of the immune system cells [44]. TLR9 was originally thought to be activated by unmethylated CpG sequences in DNA of bacterial and viral origins only [45]. It was later found that TLR9 can also be activated by unmethylated CpG sequences in fungal DNA [46]. The *A. fumigatus* DNA contains unmethylated CpG sequences, and is therefore capable of activating TLR9 and induce the production of pro-inflammatory cytokines [47]. Since TLR9 is an intracellular receptor, it was suggested that the activation would follow the release of DNA content after fungal lysis in the phagosome. TLR9 is recruited to the phagosomes that contain internalized *A. fumigatus* conidia [48]. However, TLR9 is already recruited to the phagosome merely 1h after phagocytosis of resting conidia [48]. At this stage, the internalized conidia should still be dormant and intact, and the fungal DNA is not exposed. This leaves the underlying recognition mechanism of the fungal DNA and the activation of TLR9 unresolved. Paradoxically, in vivo, TLR9-deficient mice, immunosuppressed by cyclophosphamide, had lower fungal burden than the wild-type mice upon intranasal challenge by *A. fumigatus* [49]. Consistently, in another in vivo study, the neutrophil-depleted TLR9-deficient mice was less susceptible to challenge of dormant or swollen *A. fumigatus* conidia by showing delayed mortality [50]. The expression of dectin-1 was also significantly lowered in the bone marrow-derived dendritic cells from TLR9-deficient mice 14 days post-infection following challenge of swollen conidia [50].

Taken together, the role of TLR2, 4 and 9 in host defense against *A. fumigatus* remains to be further explored.

4.2.1.2 C-Type Lectin Receptors

C-type (Ca^{2+}-dependent) lectin receptors (CLRs), including dectin-1, dectin-2, mannose receptor, Mincle, and DC-SIGN, are important in recognizing the major carbohydrate moieties in the fungal cell wall [51].

Dectin-1 is a major transmembrane receptor for β-1,3-glucan which is found mainly on myeloid cells [52–54]. Since β-1,3-glucan is the major constituent of the fungal cell wall, it was expected that dectin-1 plays a crucial role in the control of fungal infection and especially favors recognition and phagocytosis of fungal particles [37, 55]. In the case of *A. fumigatus*, dectin-1 preferentially recognizes swollen and germinating conidia, as the β-1,3-glucan is exposed on the conidial surface [56–58]. Dectin-1 contains an extracellular lectin-like carbohydrate recognition domain and a cytoplasmic tail, which is phosphorylated at the tyrosine, upon binding

with β-1,3-glucan. The phosphorylated cytoplasmic tail then can interact with spleen tyrosine kinase (SYK), which induces cellular responses, such as respiratory burst, phagocytosis and production of pro- and anti-inflammatory cytokines [54, 58, 59]. Dectin-1 induces different cytokine responses independent or in conjunction with TLR: for example, dectin-1 alone induces the production of IL-10, but requires the adaptor MyD88 for the induction of IL-8 and IL-12 [54, 56, 60].

The ligands for the other membrane-bound CLRs remained to be fully determined. Another member of the dectin family, dectin-2, has been shown recently to recognize *A. fumigatus* galactomannan, but its role in the innate response has not been really evaluated [61–63]. Mannose receptor, Mincle and DC-SIGN recognize the N-linked mannan and N-acetylglucosamine (GlcNAc) in the fungal cell wall [51, 64]. Being a polymer of GlcNAc, chitin could also be recognized by mannose receptor [65]. However, it was recently found that chitin is mainly recognized by the Fcγ receptor [66]. Further studies should be warranted to better investigate the respective roles of these polysaccharides tested alone or in association, in the immune response.

4.2.1.3 Crosstalk Between TLRs and CLRs

Some PRRs collaborate to produce a synergetic induction of immune response [67]. For instance, dectin-1, which recognizes β-1,3-glucan, interact with TLR2, which recognizes zymosan, but not β-1,3-glucan [68]. Interestingly, crosstalk between PRRs does not always lead to enhanced immune response. The CLR Mincle suppresses the antifungal immune response mediated by dectin-1 and Mincle [69, 70]. This has also been suggested to be an evasion mechanism of fungal pathogen from the host immune defense [70]. However, this has not been studied in *A. fumigatus*.

4.2.2 Humoral Pattern Recognition Receptors

4.2.2.1 Complement Components

The complement system is often considered an irrelevant immunological weapon against *A. fumigatus* for the following reasons. First, owing to the presence of the thick fungal cell wall, the membrane attack complex (MAC) of the complement system is ineffective on *A. fumigatus* [71, 72]. Second, complement deficiency in human was not found to increase the risk of aspergillosis. However, all three of the complement pathways, classical, alternative and the lectin pathway, are indeed involved in the host defense against *A. fumigatus*, through opsonization and thus, facilitation of phagocytosis [73]. Complement component C3 can directly, or through other components, such as mannose-binding lectin or immunoglobulin, deposit on the surface of the conidia and mycelia [73–78]. The exact chemical nature of the ligand(s) of the complement components on the surface

of the conidia or mycelia remain unknown. Furthermore, *A. fumigatus* can evade from the complement attack by scavenging complement regulators, Factor H and C4b binding protein [79, 80], and secreting proteases that degrades complement proteins [81]. Alp1p, a major protease secreted by *A. fumigatus*, can degrade complement proteins C3, C4 and C5 [81].

4.2.2.2 Collectins and Ficolins

Collectins (Collagen + lectin) are a group of soluble pattern recognition receptors characterized by a collagen-domain and carbohydrate recognition domain (CRD), which is responsible in the binding to the carbohydrate moiety in a Ca^{2+}-dependent manner. Collectins in the lung bind to *A. fumigatus* conidial surface as opsonins, and facilitate phagocytosis [51, 82, 83].

Surfactant Proteins

There are four surfactant proteins (SP) in human, SP-A, SP-B, SP-C, and SP-D, that are secreted by alveolar type II cells into the alveolar space [84–86]. Both SP-B and SP-C are small hydrophobic proteins that are mainly responsible in reducing surface tension at the air–liquid interface in the lungs, and their role during *Aspergillus* infection have not been studied [87]. It was only recently found that SP-C binds to bacterial lipopolysaccharides [85, 88]. Apart from that, the immunomodulatory role of SP-C is still uncertain. Meanwhile, SP-A and SP-D, which are hydrophilic proteins, are primarily responsible for the host defense against pulmonary pathogens by facilitating phagocytosis after opsonization [84, 86, 89–91]. SP-A and SP-D are not involved in the lectin complement pathway [92].

Human SP-A and SP-D bind directly to surface of dormant conidia, swelling conidia and hyphae of *A. fumigatus* [83, 93], and the responsible ligand(s) are under investigation by our group. When bound to microorganisms via its CRD region, SP-D binds to the calreticulin/CD91 complex on the surface of macrophages, via its collagenous region, which then mediates the uptake of SP-D opsonized microorganisms by phagocytes and, stimulates inflammatory response by activating NF-κB [94, 95]. Although it was found that the mortality of SP-D-deficient mice was similar to that of the wild-type mice following intranasal challenge of *A. fumigatus* conidia, under immunosuppression of corticosteroids, shorter survival duration, higher hyphal density and more tissue damage were observed in the SP-D deficient mice in comparison to the wild-type [96, 97]. This observation suggested that SP-D plays a role in immune recognition and killing, which have been however insufficiently investigated.

Compared to SP-D, the role of SP-A in the immune defense against *A. fumigatus* is less significant. Although human SP-A bind to conidial surface, the binding is reduced in the presence of extracellular alveolar lipids, while that of SP-D remained unchanged [93]. Immunosuppressed SP-A deficient mice are resistance to lethal

IPA challenge when compared to wild-type mice [97], which could be explained by the increased level of SP-D in SP-A-deficient mice [98].

At present, no evasion mechanism from the opsonization of collectins has yet been observed in *A. fumigatus*.

Mannose-Binding Lectin and Ficolins

Mannose-binding lectin (MBL) binds to the conidial surface of *A. fumigatus* [99]. Mannose-binding lectin by binding to the MBL-associated serine proteases (MASP1, MASP2, and MASP3), activate the lectin complement pathway [100]. Non-immunosuppressed MBL-knockout mice were less susceptible to systemic invasive aspergillosis [101]. It was suggested that the lack of MBL reduced the recruitment of neutrophils, which in turn leads to less tissue damage from inflammation. In contrast, in human, a deficiency in MBL is associated with chronic and invasive aspergillosis [102, 103]. The role of MBL should be revisited.

Ficolins (fibrinogen + collagen + lectin) are a family of lectins that consist of a collagen-like domain and a fibrinogen-like domain described as recognizing specifically N-acetylglucosamine (GlcNAc) [104]. There are three types of ficolins in human, ficolin-1, 2, and 3, which are secreted into the human plasma and binding to ficolin activate the lectin complement pathway [104, 105]. Ficolin-2 binds to *A. fumigatus* [74, 106, 107]. The in vitro binding of ficolin-2 to *A. fumigatus* is inhibited by the presence of GlcNAc and Curdlan (β-1,3-glucan) [107], suggesting that chitin and β-1,3-glucan are the responsible ligands. Previously, ficolin-1 and ficolin-3 were not found to bind to *A. fumigatus*. However, recently, the role of ficolin-3 in lung immune defense was discovered. Ficolin-3 indeed binds to *A. fumigatus* resting or swollen conidia in a calcium-independent manner [74, 82], suggesting that the binding does not involve the carbohydrate recognition domain. Unlike ficolin-2, which is produced in the liver, ficolin-3 is produced by type II alveolar cells and secreted into the alveolar space. Moreover, ficolin-A (the orthologue of ficolin-2 in mouse) increases the release of IL-8 (a proinflammatory cytokine and chemokine for neutrophils) [74]. Upon binding to the carbohydrate ligand on the pathogen surface, ficolins facilitate opsonization, phagocytosis and the activation of the lectin complement pathway. However, complement activation was not impaired in ficolin-knockout mice [74, 108]. This could be explained by the similar role of MBL and ficolins, that both trigger the lectin complement pathway.

Although many elements of the immune system in the anti-*A. fumigatus* response have been discovered or rediscovered in recent years, a comprehensive picture of their roles as well as the ranking of their importance in the immunocompetent, as well as immunocompromised host remains to be elucidated.

4.3 The Cell Actors

4.3.1 Airway Epithelial Cells

Airway epithelial cells (AECs) are the first type of cells to enter in contact with the inhaled conidia [109, 110]. AECs, being nonprofessional phagocytes, are capable of internalizing and killing *A. fumigatus* conidia [111, 112]. Comparing with murine macrophage cell line (J774), the internalized conidia survive longer in the human airway epithelial cell line (A549) than in the alveolar macrophages [113]. Accordingly, a small portion of the internalized conidia are not killed by the AECs, which then germinate and break the epithelial barrier [113]. The inefficient killing of the internalized conidia by the AECs is probably due to inefficient respiratory burst. In addition, the adherence of *A. fumigatus* conidia to the A549 cell line inhibits the release of IL-6, IL-8, and TNF-α, which thus inhibits the recruitment of immune cells and apoptosis of the epithelial cells [114, 115].

AECs produce antimicrobial peptides such as human β-defensins, human lactoferrin (hLF), and histatin 5, which possess some antifungal activity against *A. fumigatus* [4, 6, 109, 110, 116, 117]. The gene expression of human β-defensins hBD2 and hBD9 were found to be induced in A549 cells that are exposed to swollen conidia [116]. hBD2 displayed direct, but low antifungal activity in vitro against *A. fumigatus* [118] and does not enhance the antifungal killing activity of neutrophils [118]. However, hBD could act as chemoattractant for immune cells and activation of professional antigen-presenting cells [119].

4.3.2 Alveolar Macrophages

The resident alveolar macrophages (AMs), which are responsible of the recognition and phagocytosis of the *A. fumigatus* conidia, serve as the major immune cells in the defense of *A. fumigatus*. Upon internalization of *A. fumigatus* conidia, phagolysosomes were not acidified [18]. Dihydroxynaphthalene-melanin (DHN-melanin) on the outer layer of dormant *A. fumigatus* conidia was shown to inhibit the acidification of phagolysosome, and thus, prevent intracellular killing [120–122]. The mutant of polyketide synthase (Δ*pksP*), the enzyme responsible for the initial step in DHN-melanin formation is devoid of DHN-melanin and avirulent [123]. Interestingly, the melanin of *A. niger*, which is different from the melanin in *A. fumigatus* [124, 125], does not inhibit phagolysosome acidification [18]. The intracellular killing of *A. fumigatus* conidia in AMs is triggered by the swelling of conidia. The phagolysosome acidification and the increase in the production of reactive oxygen species (ROS) following phagocytosis is essential for conidial killing [126, 127]. Upon swelling, the outer layer of hydrophobins and melanin is shed. In the absence of inhibition by melanin, the phagolysosome acidifies. On the other hand, the absence of the outer layer of rodlet and melanin unmasks the PAMPs in the inner layer. The exposure of PAMPs

recruits PRRs to the phagosome, which triggers production of ROS, cytokines and chemokines and the recruitment of autophagy proteins, LC3 II, Atg5 and Atg7 to the phagolysosome [57, 128, 129]. Pyomelanin, another type of melanin that *A. fumigatus* produces, can also protects the fungus from ROS attack [13, 120, 130]. However, pyomelanin-minus mutant are as pathogenic as their parental strain. In addition, DHN-melanin inhibits LC3-associated phagocytosis (LAP) [123, 131, 132]. Taken together, pyomelanin and DHN-melanin permits the fungus to escape host immune defense by inhibiting phagolysosome acidification, quenching ROS and inhibiting LC3-associated phagocytosis.

4.3.3 Neutrophils

Neutrophils act as a strong second line of innate immune defense against *A. fumigatus* [133]. Upon recruitment to the alveolar space, neutrophils constitute the majority of phagocytes to eliminate the conidia [4, 134]. Even though AMs by themselves are able to eradicate resting conidia in a mouse deprived of neutrophils, neutrophils are more important than AMs in the pathogen clearance [127, 135]. Indeed, low number of neutrophils in the host (neutropenia) renders patients at great risk of IPA [2]. Moreover, molecules of *A. fumigatus* which impairs the neutrophil action are true virulence factors. Galactosaminogalactan is one of them. This polysaccharide, which is secreted by *A. fumigatus* hyphae, suppresses the recruitment of neutrophils, which then favors the fungal survival in the host [136, 137]. In addition, it induces anti-inflammatory response by the induction of interleukin-1 receptor antagonist (IL-1Ra) and reduces neutrophil recruitment [136, 138].

An important function unique to neutrophils is its capacity of attacking *A. fumigatus* hyphae, which are too large to be phagocytosed by immune cells. Neutrophils attack the fungal hyphae extracellularly by the secretion of enzymes, which degrade and permeabilize the cell wall and makes the fungus more sensitive to the neutrophil granular toxic molecules. Recently, neutrophil extracellular traps (NETs) which are mainly composed of nuclear DNA and antimicrobial proteins, have been mentioned as playing a major role against microbial pathogens [139]. Although the production of NETs is stimulated by *Aspergillus* hyphae [140], the hyphae are reported to be only slightly susceptible to the killing by NETs [141]. It is suggested that NETs only has a fungistatic effect on the hyphae and prevent further spreading of the disease [141]. This finding is in contrast to the one regarding *Candida albicans*, which is completely susceptible to the killing by NETs [142]. The fungistatic activity of NETs towards *A. fumigatus* hyphae is attributed by calprotectin, a Zn^{2+} chelator, [143]. Interestingly, it was shown recently that *A. fumigatus* hyphae generate hyphal branching upon interaction with neutrophils, which allows lower host immune interference and increases invasive growth [144].

Moreover, *A. fumigatus* seems to have other mechanisms to evade the neutrophil antagonistic action since *A. fumigatus* is less sensitive than *A. niger* for which the germination and hyphal length are more significantly reduced by neutrophils [18].

For example, fumagillin, secreted by the *A. fumigatus* hyphae, inhibits the antifungal function of neutrophils by inhibiting the formation of NADPH oxidase and reduces the degranulation [145]. Neutrophils exposed to fumagillin in vitro also demonstrated reduced rate of phagocytosis of *A. fumigatus* conidia [145]. An analysis of the differences between *A. fumigatus* and other non- or poorly pathogenic *Aspergillus* species, such as melanin structure and cell wall composition, should definitely reveal more information of the success of *A. fumigatus* as pathogen.

4.4 Perspectives

The current arsenal of antifungal agents available for the treatment of IA is mainly limited to azoles, echinocandins, and polyenes [146]. However, there are major drawbacks regarding the drug resistance, efficiency, and toxicity of these current agents [147, 148]. Furthermore, the pipeline of antifungal development has not been introducing any new classes of antifungal agents, especially since there is not a market for drugs for rare diseases such as aspergillosis. Can immunotherapy lead to the development of specific drugs or antibodies that are able to block virulence factors that allow the evasion of *A. fumigatus* from the host immune system? The past decade has witnessed a shift in paradigm of antifungal development, in which the virulence factors are targeted for drug candidates instead of essential genes [149–151]. Analysis of the transcriptomic and proteomic changes occurring after internalization of the pathogen and especially in the phagolysosome, where the fungus is inhibited in the immunocompetent host but not in the immunocompromised host, may lead to the identification of essential virulence factors in vivo. Such approach focused on the inhibition of virulence factors would exert less selective pressure on the pathogens, and thus, the chance of resistance development is lower [152]. However, such immunotherapeutic approach has not been yet undertaken with *A. fumigatus* but the situation may be difficult in the case of pulmonary aspergillosis, which occurs mainly among immunocompromised patients. The identification of monoclonal antibodies able to block hyphal emergence could be also possible. Such strategy has been proposed a few years ago [153, 154], however it has not been pursued yet. Although a lot of progresses have been obtained in recent years, the overall picture to fully understand the innate immune defense against *A. fumigatus* is missing. For example, the respective role of the different PRRs and the lack of definition of all the ligands binding to complement proteins, collectins and other PRRs are essential gaps to fill. The reasons for the ineffectiveness of NETs to kill *A. fumigatus* hyphae should also be investigated. Comparative characterization of major *Aspergillus* species may also provide valuable understanding of how *A. fumigatus* took the "throne" and became the most predominant and successful pathogen among the *Aspergillus* species. Finally, it has been reported that many cytokines and chemokines plays a major role in the defense reactions (not discussed here but see for review [155, 156]. However, the interweaving network between all these chemokines, cytokines and the defense against

A. fumigatus remains obscure. In conclusion, the early events "when *A. fumigatus* meets the man" remain enigmatic and this chapter paves the way for the direction of future exploration.

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