The Pathogenesis of *Aspergillus fumigatus*, Host Defense Mechanisms, and the Development of AFMP4 Antigen as a Vaccine

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A b s t r a c t

Aspergillus fumigatus is one of the ubiquitous fungi with airborne conidia, which accounts for most aspergillosis cases. In immunocompetent hosts, the inhaled conidia are rapidly eliminated. However, immunocompromised or immunodeficient hosts are particularly vulnerable to most *Aspergillus* infections and invasive aspergillosis (IA), with mortality from 50% to 95%. Despite the improvement of antifungal drugs over the last few decades, the therapeutic effect for IA patients is still limited and does not provide significant survival benefits. The drawbacks of antifungal drugs such as side effects, antifungal drug resistance, and the high cost of antifungal drugs highlight the importance of finding novel therapeutic and preventive approaches to fight against IA. In this article, we systemically addressed the pathogenic mechanisms, defense mechanisms against *A. fumigatus*, the immune response, molecular aspects of host evasion, and vaccines' current development against aspergillosis, particularly those based on AFMP4 protein, which might be a promising antigen for the development of anti-*A. fumigatus* vaccines.

K e y w o r d s: *Aspergillus fumigatus*, vaccine, *Aspergillus fumigatus* mannoprotein

Introduction

Aspergillus spp. is a genus of saprophytic fungi, which is widely distributed in nature. This genus plays an important role in environmental nitrogen and carbon recycling and relies on conidia to spread in the air (Krüger et al. 2015; Latgé and Chamilos 2019). Among the approximately 200 *Aspergillus* species, less than 20 are pathogenic for humans (Paulussen et al. 2017; Mead et al. 2019). *Aspergillus fumigatus* exerts a major influence on the number of pathogenic *Aspergillus* strains. Statistical data revealed that among multitudinous *Aspergillus* spp. isolates, *A. fumigatus* accounted for 50–60%, *A. flavus*, *A. terreus*, and *A. niger* each made up 10–15% of the isolates, and other uncommon *Aspergillus* spp. were less than 2% (Paulussen et al. 2017; Hoenigl et al. 2018).

Of all pathogenic *Aspergillus* spp., *A. fumigatus* with airborne conidia is a prevailing agent for human infections. The small sizes of conidia allow them to reach the lung alveoli from the natural environment effortlessly. It is estimated that humans may inhale as many as hundreds of *A. fumigatus* conidia every day (Alanio et al. 2017; Takazono and Izumikawa 2018). In healthy hosts, the inhaled conidia are rapidly eliminated by a competent immune system composed of innate and adaptive immunity. The innate immunity plays pivotal role in destroying most of the inhaled conidia in the respiratory tract by respiratory ciliary movement and proteins on the surface of epithelial cells, and in recognizing and engulfing the remaining conidia by alveolar phagocytes through surface pattern recognition receptors. Simultaneously, phagocytes also induce inflammatory chemokines and cytokines and recruit other immune cells to destroy surviving *A. fumigatus* spores and hyphae. Among them, neutrophils can prevent the formation of hyphae and kill it; monocytes can phagocytose spores to prevent fungal outbreaks. When dendritic cells phagocytose *A. fumigatus*, antigenic components are presented on the cell membrane surface to activate T

cells and B cells, initiating adaptive immunity of the body. Whereas, if the immune responses are excessively intensive, some immunological diseases such as allergic bronchopulmonary aspergillosis will occur. In immunocompromised or immunodeficient hosts, such as the patients with immunosuppressive treatment for autoimmune disease, HIV suffers, and transplantation recipients, the growth of spores and hyphae of *A. fumigatus* cannot be prevented due to the decrease of neutrophils and phagocytes. Eventually, the hyphae of *A. fumigatus* will invade human blood vessels and spread from blood to the whole body, causing multi-system infection (Filler and Sheppard 2006). *A. fumigatus* is the most lethal invasive pathogenic fungus, with the mortality from invasive aspergillosis (IA) above 50%, even up to 95% (Dos Santos et al. 2020), especially for the patients with acute leukemia and hematopoietic stem cell transplantation (HSCT) (van de Peppel et al. 2018).

Despite the wide applications of antifungal drugs, they failed to provide satisfying treatment for invasive aspergillosis patients. Notably, the clear side effects of antifungal drugs such as amphotericin B's kidney toxicity and the potential hepatotoxicity of itraconazole discouraged their clinical use. Although voriconazole had better penetration than amphotericin B and itraconazole, it might cause temporary hepatotoxicity. In addition to the side effects of antifungal drugs, drug resistance and high cost also greatly hindered the use of antifungal drugs. In response to the quest for more efficacious and safer therapeutic options, various new therapeutic drugs and new dosage forms of various therapeutic drugs are also in progress. The latest drugs, such as rifconazole and abaconazole, are being tested in various *in vitro* and *in vivo* trials (Jović et al. 2019). However, more studies still need to provide useful data on the efficacy and safety of new antifungal drugs. In consequence, the development of antifungal vaccines is highly proposed. Recently, *A. fumigatus* mannoprotein 4 (AFMP4) has been recognized as a virulence factor of *A. fumigatus* and expected to serve as an antigen for the development of anti-*A. fumigatus* vaccines. In this article, we systemically review the biological characters of *Aspergillus* spp. and the pathogenic and defense mechanisms of *A. fumigatus* to provide new strategies for the treatment of *A. fumigatus*.

Pathogenicity

Disease caused by *A. fumigatus*

A. fumigatus can cause a broad spectrum of aspergillosis ranging from mild to severe symptoms for immunodeficient or immunosuppressed patients, including allergic syndromes, noninvasive infections, and IA

(Bonnet et al. 2017; Pagano et al. 2017; Gamaletsou et al. 2018; Xiao et al. 2020). The common diseases caused by *A. fumigatus* infection are as follows: (1) allergic bronchopulmonary aspergillosis (ABPA), (2) allergic sinusitis, (3) aspergilloma, (4) necrotizing pulmonary aspergillus (CNPA), (5) cutaneous aspergillosis, and (6) IA. In ABPA, the inflammation due to *Aspergillus* infection of the lungs primarily affects the patients with asthma, cystic fibrosis, and bronchiectasis, which cause allergy symptoms such as fever, cough, wheeze, and generalized malaise. Allergic sinusitis is a noninvasive and recurrent inflammatory sinusitis with the hypertrophic sinus and nasal polyps as the manifested symptoms in patients. Aspergilloma predominantly exhibits mycelial balls in the damaged lung bronchia, pulmonary cyst, or lung cavities, which causes a typical hemoptysis symptom in severe patients, and even threatens lives. CNPA usually occurs in patients with mild-to-moderate immunosuppression, accompanied with chronic symptoms like fever, cough, sputum, anorexia, and weight loss with a duration of 1–6 months (Barac et al. 2017). Cutaneous aspergillosis is a cutaneous manifestation of disseminated *Aspergillus* infection, including erythematous-to-violaceous plaques or papules, commonly characterized by an ulcer or eschar (Sato and Tamai 2019). IA is a severe infection that significantly affects immunocompromised patients, such as those who have had an organ transplant or a stem cell transplant operation. IA can affect each organ, but sinopulmonary diseases are the most common IA symptoms, including nasal congestion and pain, fever, pleuritic chest pain, and hemoptysis.

Molecular basis of *A. fumigatus* virulence

A. fumigatus is an opportunistic pathogen that causes ∼90% of IA with very high mortality (Darling and Milder 2018). Why *A. fumigatus* dominates the human pathogenicity is confusing to clinical medical workers, which motivating scientists to explore its pathogenic mechanisms. The pathogenicity of *A. fumigatus* to the host was mainly manifested by a direct attack with the pathogen's virulent factors, the hypersensitivity response of patients, or the innate and adaptive immunity of host evoked by virulent factors during the process of germinating in the host. In recent decades, especially after *A. fumigatus* AF293 strain genome sequencing in 2005, the virulence of *A. fumigatus* was shown to be multifactorial and was related to thermotolerance, cell wall composition and maintenance, resistance to immune response, toxins, nutrient uptake during invasive growth, signaling regulation, and allergens (Darling and Milder 2018; Latgé and Chamilos 2019). Besides, many molecules or genes related to the pathogenicity of *A. fumigatus* have been found,

including galactomannan glycoprotein encoded by *afmp1*, hydrophobic protein Rod A, fumagillin, gliotoxin, helvolic acid, fumigaclavin C, asp-hemolysin, and so on. The genes and molecules associated with *A. fumigatus* virulence either were helpful for the survival of pathogens in the host, or contributed to the process of evading the immune system, such as masking the important PAMPs, inhibition of phagosome-lysosome fusion, production of antioxidants like catalase, SOD, and mannitol, or exerted multiple immunosuppressive actions on the host immunity by producing specific secondary metabolites such as gliotoxin (GT), fumagillin, actibind, and cytochalasin E.

The genes related to thermotolerance

As a thermophilic fungus, *A. fumigatus* can grow at 55°C and survive at temperatures above 75°C. This ability facilitates to thrive in dead or decayed organic matters and to infect mammalian host cells. Thus, the genes related to thermotolerance contribute to the virulence of *A. fumigatus*. Five genes have been proved to be associated with the thermotolerance of *A. fumigatus* (*thtA*, *cgrA*, *afpmt1*, *kre2/afmnt1*, and *hsp1/asp f 12*). The *thtA* gene is necessary for the growth of *A. fumigatus* at 48°C, but it is not involved in the pathogenicity of *A. fumigatus*. The *afpmt1* gene encodes for one mannosyl transferase, which is essential for the growth of *A. fumigatus* over 37°C. It was found that the *Δafmnt1* mutant was attenuated in a mouse infection model and more sensitive to azoles (Wagener et al. 2008).

Toxins

A. fumigatus produce toxins for protection against predators and competitors, and they can directly attack the host and contribute to the pathogenesis of the fungus. Many toxins are secondary metabolites of fungi. They can affect the synthesis of DNA, RNA, and proteins, or alter cell membrane and impair cellular functions. Many toxins and relevant genes of *A. fumigatus* have been studied, such as diffusible toxic substances from conidia, gliotoxin (*gliP* and *gliZ*), mitogillin (*res/mitF/aspf1*), hemolysin (*aspHS*), verruculogen, fumagillin, and the transcription factor *laeA*. Gliotoxin is the most potent toxin produced by *A. fumigatus* (Zhang et al. 2019), which can suppress macrophage phagocytosis, T cell proliferation, cytotoxic T cell response, and monocyte apoptosis (Schlam et al. 2016; Schmidt et al. 2017; Fraga-Silva et al. 2019). Gliotoxin can also inhibit the NADPH of neutrophils (Tsunawaki et al. 2004), suppress ROS production, and impair the neutrophil's phagocytic capacity (Orciuolo et al. 2007). In addition, it should be noted that the transcription factor *laeA* is a crucial regulator for secondary metabolite biosynthesis (Pfannenstiel et al. 2017), and it has been proved that *laeA* deletion in *A. fumigatus* inhibited the pro-

duction of almost all secondary metabolites containing gliotoxin (Arias et al. 2018).

Allergens

Moreover, *A. fumigatus* can produce a large number of allergens; among them 23 have their official names ranging from Asp f1 to Asp f34 (available at: http://www.allergen.org/, updated on July 11, 2019). Some allergens show toxic or enzymatic activities, which are related to the virulence. Other allergenic molecules have no virulence functions. All *Aspergillus* allergens are likely to trigger a Type I hypersensitivity response in patients and induce a high-affinity IgE antibody production. *Aspergillus* allergens can cause hypersensitivities in immunocompetent patients, such as ABPA, allergic rhinosinusitis, asthma, and aspergilloma. In immunocompromised patients, these allergenic molecules can significantly increase the risk of aspergillosis. Many *Aspergillus* allergens have been explored and developed for diagnostic purposes (Masaki et al. 2017).

Other pathogenic factors

So far, none of the pathogenic factors is unique to *A. fumigatus*, and it is necessary to further investigate why *A. fumigatus* is more pathogenic than other common conditional pathogens. Some scholars believed that, unlike other human pathogenic bacteria such as *Candida* and *Cryptococcus*, the pathogenicity of *A. fumigatus* is not caused by one or several pathogenic factors but caused by the result of its unique biological characteristics such as growth and metabolism and the joint action of multiple pathogenic factors.

Recently, based on the homology between fungal endoether glucokinase and AnmK kinase of bacterial cell wall circulatory metabolism, we proposed the hypothesis that fungal cell wall has a mechanism similar to that of bacterial cell wall circulatory metabolism, which plays a role in the growth and reproduction of fungi. Whether this hypothesis is correct and related to the pathogenicity of *A. fumigatus* needs to be further confirmed by research.

Defense mechanism against *A. Fumigatus*

Host immunity to *A. fumigatus*

Innate defense immunity

Anatomical barriers. At the entry point of airborne conidia, the upper respiratory tract's airway epithelium is the first defensive line of innate host immunity against *A. fumigatus*. As an airway epithelium cell, the mucous secreting cell can secrete mucus to trap inhaled conidia. Another airway epithelium cell, a ciliated cell, can drive the trapped conidia to the oropharyngeal junction

(van de Veerdonk et al. 2017). In this way, a significant number of *A. fumigatus* are expulsed from the lung. The respiratory epithelium can also secrete some peptides or enzymes to combat *A. fumigatus*, indicating that chitinase produced by epithelium can damage chitin on the cell wall of *A. fumigatus* (Garth et al. 2018).

Professional phagocytes and classical signaling pathways. The dominant role of phagocytes defense against *A. fumigatus in vivo* and *in vitro* has been reported (Liu et al. 2017; Almeida et al. 2019; Mackel and Steele 2019). The primary phagocytes responsible for the phagocytosis of *A. fumigatus* are alveolar macrophages (AM) and neutrophils.

In the lung of the immunocompetent host, certain soluble recognition receptors produced by alveolar macrophages such as Pentraxin 3 (PTX3) and surface protein-D (SP-D) can immediately bind to the inhaled conidia of *A. fumigatus,* and enhance the phagocytosis of alveolar macrophages (Smole et al. 2020). Alveolar macrophages then recognize and swallow the conidia through the TLR2/4 and Dectin-1. Toll-like receptors (TLRs) are type I membrane receptors that function in recognition of PAMPs and an intracellular TLR domain required for downstream signaling. TLRs can recognize pathogens and activate transcription factors such as NF-κB, which mediate the expression of inflammatory cytokines and chemokines (Anthoney et al. 2018). Some studies implicated the membrane receptors TLR2 and TLR4 were the crucial recognition components for host defense against *A. fumigatus* (Dai et al. 2019). An essential role for TLR2 and TLR4 in cytokine production against *A. fumigatus* has been established in many *in vitro* studies (Briard et al. 2019; Gupta et al. 2019). Dectin-1 is a type II transmembrane protein, which is highly expressed in macrophages, neutrophils, and DCs. Dectin-1 can recognize β-glucan on germinating conidian but cannot identify the resting conidia, allowing macrophages to differentiate the various forms of *A. fumigatus* (Li et al. 2019; Dutta et al. 2020). Alveolar macrophages capture conidia leading to a proinflammatory response accompanied by the secretion of many cytokines and chemokines, including TNF-α and CXCL2 (Chemokine (C-X-C motif) ligand 2), which are essential activators for neutrophil recruitment (Guo et al. 2020). The conidia escaped from the phagocytosis by alveolar macrophages continue to germinate and spread. Proinflammatory factors derived by alveolar macrophages and epithelial cells recruit neutrophils to the infection site. Neutrophils perform an effective elimination of the germinating conidia and hyphae.

In the immunocompetent lung, conidia are immediately trapped by the soluble recognition receptors (PTX3, SP-D), promoting conidial phagocytosis by alveolar macrophage (AM). AM also captures conidia through TLRs and Dectin-1, leading to a proinflammatory response. The escaped conidia continue to germinate and penetrate through the alveolar epithelial cells. Neutrophils employ the processes of NET formation, degranulation, and lactoferrin production to inactivate germinating conidia and hyphae. Dendritic cells phagocytose, and process germinated conidia for antigens presentation to T cells, and finally activate an adaptive immune response to *A. fumigatus* (Garth and Steele 2017).

Mechanism of innate immune cells removing A. fumigatus. The mechanism includes phagocytosis, reactive oxygen species (ROS) generations mediated by nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), lactoferrin production, and neutrophil extracellular traps (NETs) formation (Schoen et al. 2019; Souza et al. 2019; Shopova et al. 2020). ROS production responds to swollen conidia, but not resting conidia, through NADPH oxidase activation (Ferling et al. 2020; Khani et al. 2020). It has been demonstrated that the ROS-producing complex plays a crucial fungicidal role during *A. fumigatus* infection (Shen et al. 2016). NETs are networks of extracellular fibers, mainly composed of DNA from neutrophils. The NET formation is induced by a variety of proinflammatory mediators such as IL-8. It is significant for defense against large pathogens, such as hyphae of *A. fumigatus* (Li et al. 2020). It has been demonstrated both *in vitro* and *in vivo*, NET formation is dependent on NADPH oxidase and ROS generation (Khan et al. 2019; Ravindran et al. 2019). In addition, dendritic cells (DCs) play a well-established role in the host defense against *A. fumigatus*. Immature DCs can phagocytose conidia and hyphae through PRRs and present the processed antigens of *A. fumigatus* to host T cells, leading to the activation of adaptive immune responses (Wang et al. 2017).

Adaptive immunity mechanism

The elimination of the daily-inhaled conidia mainly depends on the innate immune response, but the treatment for serious *Aspergillus* infections relies on the cooperation of the adaptive immune system, which responds to the signaling generated by innate immunity. Lymphocytes T and B represent the two main parts of the adaptive defensive system.

Role of T cells in adaptive immunity. The T cell immunity system interacts with the innate immune response in many ways. For instance, DCs recruit at the infection sites can load and migrate the *A. fumigatus* antigen to lymph nodes, leading to T lymphocytes' activation (Wang et al. 2017). CD4+ T cells are the dominating organizer, which play a major role in antifungal immunity. These activated T cells are able to invoke phagocytes or restrict the immune response. The initiating of CD4+ T cell immunity occurred between TCR and its cognate antigens on the DC cells. The excessive

inflammatory response secreted by the activated T cells can induce the naïve T cells to differentiate into CD4 T help (Th) subsets while damaging the incident tissues and contributing to the invasive infections of *A. fumigatus*. Th1 response was the predominant cell type that involved the protective immune response to the host through the production of pro-inflammatory cytokines such as IFN-γ, IL-2, IL-12, and TNF-α. In contrast, Th2 immune response was associated with the germination of fungal and exacerbation of disease as well as induced the alternatively activated macrophages in the defense against *A. fumigatus*. The balance between Th1 and Th2 subset determined the quality and outcome of host immune responses. It was also found that Th17 played multiple roles in clearing infections by participating in the production of proinflammatory genes and antimicrobial peptides, recruitment, and activation of neutrophils (Pathakumari et al. 2020). T cell immunity against fungal infections primes Th1 type response (Shenoy et al. 2017). However, in patients with aspergillosis, the predominance of Th2 T cells' immune response was conducted to exacerbate disease (Dewi et al. 2017). Besides, DCs contribute to a damaging inflammatory response by stimulating the Th17 cells and producing IL-23 (Movahed et al. 2018).

Humoral immunity to A. fumigatus. The function of *Aspergillus*-specific antibodies in immunocompromised patients with IA has been investigated (Boniche et al. 2020). Early researches indicated that the antibody responses failed to provide effective protection against IA or played only a minor role in fighting aspergillosis (Cutler et al. 2007). However, recent research reported that β-1,3-glucan specific antibodies could not only inhibit *Aspergillus* hyphae but also protect CD2F1 mice against *Aspergillus* challenge (Matveev et al. 2019). Although it is generally acknowledged that T cells immunity plays a vital role in combating fungal aspergillosis (Diaz-Arevalo and Kalkum 2017), there are a variety of ways, such as opsonization, complement activation, and virulence factors neutralization, in which antibodies affect T cells response and suppress the growth, adherence, and germination of fungi (Liedke et al. 2017; Ulrich and Ebel 2020).

Progress of anti-*A. fumigatus* vaccines development

Despite the wide applications of antifungal drugs, they failed to provide satisfying treatment for IA patients. The therapy with antifungal drugs is often associated with side effects, drug-resistance, and high costs. To overcome these disadvantages, the development of alternative methods, including antifungal vaccines, is highly desirable. Antifungal vaccine studies' primary tool is the employment of fungal particulate forms, homogenates, or recombinant proteins. Some studies revealed that the immunization with conidia, mycelia extracts, or fungal culture filtrates induced effective protection against *Aspergillus* infections (Muthu et al. 2018; Pérez-Cantero et al. 2019). The heat-killed mutant strain was also reported to be a broad-spectrum fungal vaccine that induced host protection against common invasive fungal infections in both immunocompetent and immunocompromised hosts (Wang et al. 2019). However, the crude extracts commonly consist of abundant fungal components as various carbohydrates, nucleic acids, or even some toxins (Shishodia et al. 2019). Thus, vaccination using purified recombinant antigenic protein or peptide-based vaccine (Da Silva et al. 2020) is a more popular method in antifungal vaccine studies, whereas the possibility of potential severe anaphylaxis remains.

Obstacles in vaccine development

Although recently there is some progress published on the study of vaccines against *A. fumigatus* (Chauvin et al. 2019; Khani et al. 2020), several significant obstacles remain to be overcome for producing effective vaccine (Levitz 2017). First of all, since *A. fumigatus* is an opportunistic pathogen and most invasive infections appear in the immunocompromised population, the induction of an effective adaptive immunity in such individuals is a real challenge. One of the feasible measures is the prophylactic vaccination, especially in target population, such as the patients waiting for bone marrow transplant and the patients before the treatment with immunosuppressive agents. Another major problem in developing a vaccine against *A. fumigatus* is related to the fungus' molecular complexity. The extract of *A. fumigatus* is a mixture containing up to 200 different proteins, glycoproteins, and compounds of low molecular weights. In addition, safety issues should also be well addressed because a wide range of allergic diseases correlates with *Aspergillus* allergens. The potential of activating an adverse immunoreaction is also an intractable problem (Dewi et al. 2017).

Adaptive immunity induced by anti-*A. fumigatus* vaccines

Currently, some *A. fumigatus* allergens were identified based on their reactivity to patients' antibodies. Some of these allergens are not effective antigens for vaccine development because they primarily induce Th2 cell response but cannot provide adequate protection against *Aspergillus* infection. Nevertheless, a recombinant allergen of *A. fumigatus* – Aspf3 was confirmed to induce protective response when presented mixed with TM adjuvant in a murine inhalation model (Namvar et al. 2015). It was also reported that various yeast genera could activate innate CD8+ T lymphocyte response and generate broad antifungal protection (Bazan et al.

2018). It suggested yeasts as a potential antifungal vaccine candidate to activate antigen-specific CD8+ T cell responses effectively.

For a long time, most antifungal vaccine research intended to activate memory T lymphocytes and raise a Th1 type immune response that would produce some favorable cytokines to enhance phagocytosis or T cell killing (Upadhya et al. 2016). Most studies suggested that the protective antifungal reactions induced by vaccines are cell-mediated immune responses. However, some recent investigations focused on protective antibodies. One β-glucan conjugate vaccine was shown to provide good protection against *Candida albicans* and *A. fumigatus* (Catellani et al. 2020). The serum of vaccinated mice significantly inhibited the growth of fungus hyphae. The mechanism of the β-glucan conjugate vaccine might involve specific anti-β-glucan antibody (Matveev et al. 2019).

Optimizing vaccination schedules with *A. fumigatus* mannoprotein (AFMP)

The cell wall of fungi is primarily defensive to a hostile environment (Ruiz-Herrera and Ortiz-Castellanos 2019). Except for physical protection, one central role of the fungus cell wall is the interaction with the hosts. Therefore, the cell wall components are usually the targets to be attacked by the immune cells in hosts. The cell wall of *A. fumigatus* is mainly composed of polysaccharides and proteins. The polysaccharides consist of glucan, mannose, and chitin, which constitute a three-dimensional network. β-(1,3)-glucan branched with β-(1,6)-glucan forms the wall's skeleton. Chitin, a polymer of N-acetylglucosamine is covalently linked to β-glucan. Several proteins of the cell wall are mannosylated. The role of some mannoprotein has been investigated and suggested as antigenic determinants for serodiagnosis.

Mannoproteins (MPs) are natural glycoconjugates expressed mainly on the fungal surface and released into the culture medium during fungal growth. MPs have been implicated as important antigens involved in the induction of T cell-mediated immunity (Schülke 2018; Paulovičová et al. 2019). Therefore, MP may have potential use as an immunomodulator in patients at high risk of IA.

The *afmp* genes were reported to encode some cell wall MPs of *A. fumigatus*. Among them, *afmp1* encodes a protein-AFMP1 with 284 amino acids, which contains some similar sequences present in MP of *Penicillium marneffei* (Muszewska et al. 2017). AFMP2 also includes a few domains of MP1 (Woo et al. 2018). Remarkably, specific AFMP1 and AFMP2 antibodies were found in the aspergillosis or aspergilloma patients during *A. fumigatus* infections (Woo et al. 2018). More-

over, recombinant AFMP1 protein was also applied in the ELISA assay to detect specific AFMP1 antibodies in hosts, which greatly contributes to the rapid diagnosis of *A. fumigatus*-related aspergillosis (Woo et al. 2018). Based on this research, another two *A. fumigatus* MPs, AFMP3, and AFMP4 proteins were discovered during BLASTP searching conserved sequence domains of AFMP2 (Woo et al. 2018). Furthermore, it was demonstrated that AFMP1 and AFMP4 monoclonal antibodies had been generated and used to develop two ELISA methods to detect AFMP1 and AFMP4. These antigen-capture ELISA methods can rapidly and specifically detect AFMP1 or AFMP4 in the cultures of *A. fumigatus* without the cross-reactivity with other pathogenic *Aspergillus* species (Woo et al. 2018).

In MP1 and AFMP1~AFMP4 proteins, there is a putative signal peptide at the N-terminal site, which instructs secretory proteins to the endoplasmic reticulum route (Woo et al. 2018), as well as several homologous conserved domains detected through phylogenetic analysis. Considering that the virulence role of *Penicillium marneffei* mannoprotein 1 (MP1) has been confirmed (Woo et al. 2018), AFMPs may contribute to the virulence of *A. fumigatus* likewise. Our collaborators compared the difference in virulence between wild *A. fumigatus* and various afmp mutants (Woo et al. 2018). The results showed that among the four afmp1-afmp4 single knockdown mutants, the virulence of *A. fumigatus* distinctly decreased only in the afmp4 mutant. The mice infected by the conidia of afmp1-afmp3 single knockdown strains did not distinguish the survival rates in the mice challenged with conidia of wild type *A. fumigatus*. It implied that afmp1, afmp2, afmp3 might not be the crucial toxic factors, whereas afmp4 is very likely to be a decisive virulence factor for *A. fumigatus* and may be used as a promising antigen for the development of vaccines against *A. fumigatus*.

Conclusions

The multifactorial virulence factors and complex pathogenic mechanism of *A. fumigatus* put forward higher requirements for the prevention and control of fungal. Advances in the understanding of pathogenicity and host immune response to *A. fumigatus* would be conductive to propose new strategies for antifungal vaccines. The AFMP4 protein of *A. fumigatus,* which has been identified at the molecular levels, might serve as a promising candidate antigen for developing vaccines against invasive aspergillosis. Alternatively, a pan-fungal vaccine with a broader antifungal spectrum derived from the conserved fungal cell surface epitopes might be the most promising antifungal vaccine in the future. Also, several studies of animal models, adjuvants, and

immunomodulators would provide novel strategies for the design of antifungal vaccines. Nevertheless, with antifungal vaccines' progress, these vaccines' efficacy and efficiency need to be improved.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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