REVIEW

Current trends to control fungal pathogens: exploiting our knowledge in the host–pathogen interaction

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Abstract: Human fungal infections remain a major challenge in medicine. Only a limited number of antifungal drugs are available, which are often related to severe adverse effects. In addition, there is an increased emergence related to resistant strains, which makes imperative to understand the host-pathogen interactions as well as to develop alternative treatments. Host innate and adaptive immunity play a crucial role controlling fungal infections; therefore, vaccines are a viable tool to prevent and treat fungal pathogens. Innate immunity is triggered by the interaction between the cell surface pattern recognition receptors (PRRs) and the pathogen-associated molecular patterns (PAMPs). Such an initial immunological response is yet little understood in fungal infections, in part due to the complexity and plasticity of the fungal cell walls. Described host cell–fungus interactions and antigenic molecules are addressed in this paper. Furthermore, antigens found in the cell wall and capsule, including peptides, glycoproteins, glycolipids, and glycans, have been used to trigger specific immune responses, and an increased production of antibodies has been observed when attached to immunogenic molecules. The recent biotechnological advances have allowed the development of vaccines against viral and bacterial pathogens with positive results; therefore, this technology has been applied to develop anti-fungal vaccines. Passive immunization has also emerged as an appealing alternative to treat disseminated mycosis, especially in immunocompromised patients. Those approaches have a long way to be seen in clinical cases. However, all studies discussed here open the possibility to have access to new therapies to be applied alone or in combination with current antifungal drugs. Herein, the state of the art of fungal vaccine developments is discussed in this review, highlighting new advances against *Candida albicans*, *Aspergillus fumigatus, Cryptococcus neoformans, Paracoccidioides brasiliensis*, and *Sporothrix* spp.

Keywords: anti-fungal immunity, candidiasis, aspergillosis, cryptococcosis, paracoccidioidomycosis, sporotrichosis

Introduction

For a long time, innate immunity was considered as a redundant and dispensable line of defense; however, it is now known that despite the lack of specificity, like that of the adaptive immunity, it can distinguish self- from non-self-elements and can activate adaptive mechanisms by the provision of specific signals. When interacting with fungal cells, the elements of the immune system are forced to face unique challenges, by recognizing conserved molecular structures on the pathogen surface, known as pathogen-associated molecular patterns (PAMPs), via conserved transmembrane or soluble receptors, named pattern recognition receptors (PRRs). The

best-defined receptors involved in fungal recognition are the Toll-like receptors (TLRs) and the C-type lectin receptors (CLRs).[1] Upon engagement of PRRs with PAMPs, immune cells produce humoral mediators (inflammatory cytokines, chemokines, or complement elements) or uptake the fungal cell, to eliminate the pathogen and to initiate the adaptive responses.[1–3] Epithelial cells, neutrophils, macrophages, monocytes, and dendritic cells (DCs) are the main innate immune cells in establishing a protective immune response.[2,3]

The most important and better characterized TLRs participating in fungal recognition are TLR2 and TLR4. Several studies have demonstrated that TLR2 activation tilts the host immunity toward an anti-inflammatory Th2-based response, with the induction of macrophage-deactivating cytokines, such as interleukin-10 (IL-10). On the contrary, activation of TLR4 induces a Th1-based response, with the secretion of pro-inflammatory cytokines.[2,4] Nevertheless, it has been demonstrated that TLR2 can produce a pro-inflammatory response, although weaker than that mediated by TLR4.[5] One of the first evidence suggesting a key role of these receptors in the establishment of a proper antifungal response comes from the study of the *Candida albicans*-immune cell interaction.[6] Mice with a deficient *TLR4* gene had an impaired recruitment of neutrophils to the infection site and an increased probability to develop disseminated candidiasis. These defects were mediated by a decrease in the release of the keratinocyte-derived chemokine and the macrophage inflammatory protein 2.[6] It has also been demonstrated that genetic variations in TLR1 are associated with an increased risk of candidiasis.[7] Another important role of TLRs in the antifungal immune response comes from the study of immunity against *Aspergillus*. Both TLR2 and TLR4 are able to recognize conidia and hyphae of *Aspergillus fumigatus* and *Aspergillus niger* and are crucial for neutrophil stimulation during infection. *A. fumigatus* conidia are recognized by TLR4 and TLR2, inducing the production of pro-inflammatory cytokines, while hyphae are recognized only by TLR2, resulting in the secretion of IL-10.[2] In addition, the pro-inflammatory effect of TLR4 has been shown to be protective against invasive aspergillosis, as data show an increased susceptibility to infection in *tlr4*$^{-/-}$ mice.[4,5] Additional studies have demonstrated that a better recognition of *A. fumigatus* needs germination of conidia. Resting conidia are unable to induce cytokine production by macrophages, but swollen conidia and germ tubes can be recognized by Dectin-1 and TLR2.[8]

It is now well-established that engagement of TLR2 and TLR4, and activation of the MyD88-dependent signaling pathway, plays a pivotal role in cytokine secretion and activation of the phagocytic process,[2] but they are insufficient to promote a protective immune response, suggesting the participation of more components during the recognition of fungal pathogens.

The cell wall and the capsule are essential structures in the fungus–host interaction, since they are the first points of contact with the host surfaces, and several fungal polysaccharides have been identified as PAMPs recognized by PRRs, such as the CLRs.[1,9] These lectins are transmembrane proteins that control signaling processes, microbicidal activity, and phagocytosis and are involved directly in the host innate response, since fungal cell surfaces are carbohydrate-rich structures.[2] One of these receptors, Dectin-1, is a primary non-opsonic receptor for phagocytosis of soluble and particulate β1,3-glucan and can contribute to the recognition of this carbohydrate already opsonized.[4,7] Dectin-1 is able to mediate specific recognition of β1,3-glucan found in the cell walls of several fungi such as *Candida*, *Aspergillus*, *Pneumocystis*, *Sporothrix,* and *Coccidioides*.[1,10,11] In some cases, Dectin-1 and TLR2 can collaborate to increase pro-inflammatory cytokine production.[4] Fungal recognition by Dectin-1 depends on the exposure of β1,3-glucan at the surface of the wall, which can vary among different types of fungi and different morphologies.[10] Dormant *Aspergillus* conidia are not able to activate Dectin-1, given that this morphology does not express β1,3-glucan on the cell surface. After germination, β1,3-glucan is exposed and can be detected by Dectin-1.[7] Although mannans can function as immune evaders, several host receptors are able to recognize them and enhance host immunity.[12]

Dectin-2 and Dectin-3 recognize α-mannan and can form heterodimeric structures that provide high affinity to the binding of mannans to activate intracellular signaling cascades.[12,13] Dectin-2 lacks intracellular signaling motifs and therefore has to associate with other receptors to be able to transduce signals.[8,12] All three Dectins are responsible for the induction of a Th17 response, a key response in defense against fungal pathogens.[13] The macrophage inducible C-type lectin (Mincle) is a type II transmembrane receptor that binds α-mannosyl residues but not mannans, indicating recognition of terminal α-mannoses.[13] It has been demonstrated that Mincle binds to *C. albicans* and *Saccharomyces cerevisiae* but also recognizes *Malassezia* spp. In experimental models of *C. albicans* infection, mice lacking Mincle expression showed higher fungal loads in kidneys and impaired production of tumor necrosis factor-α (TNFα) by macrophages.[7,8,13] Mannose receptor (MR) is a type I transmembrane protein that recognizes mannose, fucose, and N-acetylglucosamine

residues and can be expressed on the cell surface and as a soluble form released during proteolytic cleavage.[8,12,13] It has an important role during phagocytosis, due to not only particle binding but also signaling by its intracellular portion able to couple detection of particles to phagocytosis activation.[13] In macrophages, it recognizes fungal mannans and mediates recognition and phagocytosis of *Candida* spp.[14] In addition, DCs can internalize and process mannoproteins through this receptor, leading to maturation and activation.[12] In collaboration with TLR9 and NOD2, MR can recognize chitin particles, leading to the production of IL-10 and establishing an anti-inflammatory process.[15] However, it has been demonstrated that MR can be dispensable for immunity to several fungi, given that mannans can be recognized by other receptors, such as Dectin-2 and TLR4.[8] The DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) receptor can bind *N*-linked mannans and mannose-containing oligosaccharides. It functions as an endocytic receptor and is expressed on professional presenting cells.[12] This receptor, as well as MR, is activated primarily by IL-4 and is associated with a Th2 response.[2] It has been found to participate in the phagocytosis of *Candida* and *Aspergillus* spp.[5]

Recently, the melanin-sensing CLR (MelLec) has been identified. This protein interacts with the naphthalene-diol unit of 1,8-dihydroxynaphthalene-melanin found on the surface of conidia from *A. fumigatus* and other melanized fungi. MelLec is found on the surface of endothelial and myeloid cells and is required for the protection against disseminated aspergillosis.[16]

The immunogenic ability of fungal components

During the recognition of a fungal pathogen, macrophages and neutrophils play a key role. Upon PRR–PAMP interaction, downstream signaling pathways trigger the induction of antimicrobial mechanisms and the release of proinflammatory cytokines, to stimulate and attract other leukocytes to the site of infection.[15,17] IL-10 and IL-17 are key cytokines for the defense against fungi. IL-17 is in charge of stimulating granulopoiesis and neutrophil recruitment, essential for the Th17 response. Fungal cell wall components, such as *C. albicans* mannans and β-glucans, induce prostaglandin E2, an important proinflammatory mediator for the Th17 response, via MR and Dectin-1, respectively. On the contrary, IL-10 is a Th2-derived cytokine that shifts the balance toward anti-inflammatory responses. Increased IL-10 production exacerbates *Candida* infection in mice, due to a downregulated Th1 antifungal response.[1]

In fungal cells, sphingolipids have several roles in signal transduction, cell cycle, apoptosis, and pathogenesis. *C. neoformans* and other fungal pathogens are able to produce antigenic glycosphingolipids (GSLs), such as glucosylceramide (GlcCer). The immunogenicity and clinical significance of these GSLs have been demonstrated in several fungal infections, such as cryptococcosis, aspergillosis, histoplasmosis, paracoccidioidomycosis, and chromoblastomycosis. Patients with these mycoses show high levels of immunoglobulin G (IgG1) IgG1 to GlcCer, and these antibodies are able to inhibit *Cryptococcus* growth and budding.[18]

Vesicles have been recognized as important structures related to pathogen virulence and modulation of the host immunity. Exosome-like vesicles containing virulence factors and antigens have been characterized in fungi, such as *C. neoformans*. These vesicles can carry ergosterol, GlcCer, and glucuronoxylomannan (GXM). GXM is the *Cryptococcus* major capsular polysaccharide and participates in the pathogenesis of the fungus, by diminishing NO production and increasing IL-10 and TGF-β secretion as part of a depressive effect on the immune system.[1]

Little is known about specific antigens in *A. fumigatus* that are able to induce an antifungal T-cell response. A positive T-cell response toward some *Aspergillus* antigens was seen with an analysis of 22 patients with invasive aspergillosis. Pep1, Crf1, Gel1, Sod1, α1,3-glucan, and β1,3-glucan correlated with a good disease outcome, indicating the existence of protective epitopes within these proteins. Nevertheless, only one specific epitope has been reliably used to expand specific T cells in vitro, the p41 peptide found within the Crf1 protein.[19]

Della Terra et al developed a subcutaneous murine model of sporotrichosis, mimicking the horizontal and zoonotic transmission of the mycosis. They analyzed the protein secretion profiles of 11 isolates of *Sporothrix brasiliensis* and saw that IgG antibodies produced by infected mice recognized 17 proteins ranging from 22 to 130 kDa in the whole cell extract and 38 to 100 kDa in the exoantigens. There were two immunodominant molecules detected in almost 100% of the cases, Gp70 and a 100kDa protein. A high level of cross-reaction between *Sporothrix schenckii* and *S. brasiliensis* antigens was detected, supporting the idea that the epitopes are conserved in different species of the clinical clade.[20]

Several fungal pathogens, such as *Fonsecaea pedrosoi*, *Paracoccidioides brasiliensis*, *A. fumigatus*, *C. neoformans*, and *S. schenckii*, express sialic acids on the cell surface. Sialic acids have important role in fungal pathogenesis,

participating in adhesion to host surfaces and protection from the immune response. Sialic acid-depleted cells from *C. neoformans* and *S. schenckii* are more susceptible to uptake by phagocytic cells, while removal of this sugar from *F. pedrosoi* surface increased the ability of these cells to interact with neutrophils.[21] On the contrary, when sialic acids are removed from *A. fumigatus* conidia, the uptake by pneumocytes and macrophages is reduced.[22]

From this information, it is clear that fungal elements are recognized by the host immunity, and some of them can be associated with a protective immune response. This has stimulated the field to search for antigens that can be used to develop vaccines against fungal pathogens. In the next sections, we summarize the current knowledge in this field.

Vaccine developments against *C. albicans*

Due to the increased drug resistance in fungi, it is necessary to find alternatives to fight against fungal diseases. CRM45 and CRM197, two diphtheria toxin-related proteins, named cross-reacting materials (CRMs), have attracted the attention in studying the properties of chimeric toxins or new vaccine development.[23] A glycoconjugate (Lm-CRM197) composed of β1,3-glucan polysaccharide and diphtheria toxoid CRM197 has been used as a preventive vaccine against both systemic and vaginal candidiasis in mice and rats, respectively. The protein coupled to laminarin converts this polysaccharide to immunogenic, which induces anti-β-glucan antibodies in both mice and rats. The antibodies, mostly of the IgG class, were essential for anti-*Candida* protection.[24] The first attempt to develop a *Candida*-derived ribosomal vaccine was proposed by Levy et al, where *C. albicans* ribosomal fractions containing both RNA and protein were injected subcutaneously in mice. Then, the animals were challenged either intraperitoneally or intravenously (IV) with live yeast, and 30 days after the challenge, those mice that received the ribosomal fraction increased their survival from 46% to 90% compared with 14% to 38% of controls. Those mice immunized with the ribosomal fraction and IV challenged had a survival rate of 22%–67%, whereas none of the unimmunized mice survived.[25] Another immunogenic determinant of the *C. albicans* cell wall is the phosphomannan complex (PMC). Vaccinated mice with liposome-encapsulated PMC developed longer survival time when were challenged with the fungal yeast than control groups. Likewise, if PMC is coupled with BSA, the protection against *C. albicans* is enhanced in this model of infection.[26,27] Furthermore, mice vaccinated subcutaneously with *C. albicans*-derived mannan or mannan-BSA conjugate showed a mannan dose-dependent induced protection, improved 40-fold by the conjugation of BSA to the glycan.[28]

The cell wall components of *C. albicans* such as proteins are crucial for virulence and pathogenicity. Such is the case of Als3, a hyphal-specific Glycosylphosphatidylinositol-anchored cell wall protein and a member of the *C. albicans* agglutinin-like sequence family. These proteins have several biological functions, namely cell growth, biofilm formation, cell adhesion, host invasion, and iron acquisition.[29] Thus, Als3 has become an important target to design vaccines and antibodies to control candidiasis. The mice vaccinated with rAls3p-N, a vaccine produced in *S. cerevisiae* using the N-terminal domain of Als3, showed 50% reduced mortality after the challenge with *C. albicans* compared with 100% of lethality on the control group.[30] Another safe vaccine proved against the same fungus is NDV-3, which contains Als3p-N plus a six-His tag. A significant feature of this vaccine is the capacity to cause memory B- and T-cell immune responses. The efficacy of this vaccine on vulvovaginal candidiasis model depends on the participation of both B and T cells.[31,32] Meanwhile, protective antibodies associated with Als3p have been developed to protect against *C. albicans*. The 3-A5 and 113 are monoclonal antibodies (MAbs) produced in *Pichia pastoris* and *S. cerevisiae*, respectively. The first MAb 3-A5 was against a fragment representing amino acids 18–329 of the N-terminus of Als3, while MAb 113 was against the N-terminal 433 amino acids of the same protein. Both antibodies are able to bind specifically to the hyphae, but not yeast cells, and avoid *C. albicans* adhesion to vascular endothelial cells and buccal epithelial cells.[33] Another MAb, C7, was produced in BALB/c mice by intraperitoneal injection of *C. albicans* high-molecular-weight stress mannoprotein recognized by salivary secretory immunoglobulin A. MAb C7 is able to recognize >200 kDa mannoprotein as epitope. In addition, MAb C7 interferes with the *C. albicans* iron uptake pathway.[34–36] The MAb 3D9.3 was prepared using a specific fraction of germ tube of *C. albicans* and immunizing BALB/c mice. This antibody is able to avoid *C. albicans* adhesion to both human buccal epithelial and vascular endothelial cells. Moreover, MAb 3D9.3 can discriminate between *C. albicans* and *Candida dubliniensis*. With this feature, this antibody can also be used as a diagnostic tool to differentiate between both species.[37] The cell wall proteins Als3 and Hyr1 were recognized by MAb 2G8, an anti-β-glucan antibody IgG2b. Using MAb 2G8 in human epithelial Hep-2 cells, the adherence of hyphae to host cells

decreased by 45% compared with controls, and the fungal burden in both vagina and kidney were also reduced. It is noteworthy that this MAb recognized not only *C. albicans* but also *A. fumigatus* and *C. neoformans*.[38] A recombinant human antibody, scFv3, is a single-chain variable fragment specific for *C. albicans* Als3. scFv3 can avoid adherence to either epithelial or endothelial human cells.[39] During the infection process, *C. albicans* express several proteins, and from them, hyphal wall protein-1 (Hwp1), enolase (Eno1), phosphoglycerate kinase (Pgk1), glyceraldehyde-3-phosphate dehydrogenase (Gap1), fructose-bisphosphate aldolase (Fba), and methyltetrahydropteroyltriglutamate (Met6) are highly produced during pathogenesis.[40] Peptides closer to the N-terminal end of these proteins were conjugated to a β-mannan trisaccharide epitope. All the glycopeptides, except β-(Man)$_3$-Pgk1, protected the mice against candidiasis; some of them even confer 80%–100% survival throughout a 120-day post-challenge observation period. In addition, the fungal burden on kidneys was significantly reduced or not found.[41]

Vaccine developments against *A. fumigatus*

Aspergillus spp. is one of the main fungal pathogens responsible for the increased number of deep-seated mycosis in immunocompromised populations. Since glycans are the main components of the fungal cell wall, and they are absent in the host cells, an obvious approach for immunization was to develop a glycoconjugate vaccine, as mentioned earlier. One of the first approaches generated was Lm-CRM197 that showed protection against *C. albicans* and *A. fumigatus*.[24] In a similar manner, purified cell wall α- and β1,3-glucans have been used as immunogens, and protection has been observed after intranasal vaccination.[42] From a different point of view, development of MAbs seems to be a suitable strategy for antifungal therapy, as already demonstrated for *C. albicans*. The in vivo protective efficacy of the MAb A9, an IgG1 directed against a peptide antigen found in hyphae and swollen conidia of *A. fumigatus* cell wall, was evaluated in a murine model of invasive aspergillosis with enhanced survival times.[43]

It has been demonstrated that subcutaneous immunization of corticosteroid-immunosuppressed mice using recombinant fungal allergen Asp f3 was protective against invasive pulmonary aspergillosis, by probably inducing a cellular immune response that leads to the activation of lymphocytic cells upon infection, thus restoring corticosteroid-suppressed phagocytes to clear fungal cells.[44] In addition, a wide set of recombinant proteins including Pep1, Crf1, and Gel1 are able to activate Th-cell responses in mice and humans.[42]

Another approach for immunization against aspergillosis has been the use of the inactivated whole fungal cell, such as heat-killed *S. cerevisiae* yeasts, which has been proven to be protective when administered subcutaneously to mice 2 weeks prior to the intravenous infection with *A. fumigatus*. The protection was potentiated by using alum as an adjuvant.[45] Previously, it had been already demonstrated that live or heat-killed *A. fumigatus* cells, as well as crude culture filtrates intranasally inhaled, were able to develop local and peripheral protective Th1 responses in a mouse model of pulmonary aspergillosis, mainly mediated by antigen-specific CD4+ T cells proficient to confer defense upon adoptive transfer to naïve recipients. Interestingly, after drug-induced immunosuppression, only live *A. fumigatus* or the crude filtrate, but not heat-killed fungal cells, achieved prolonged survival in mice.[46] A successice study proved that mice immunized with live conidia induced interferon-γ (IFN-γ) producing fungus-specific CD4+ T cells, unlike heat-killed conidia that mainly induced *A. fumigatus*-specific CD4+ T cells producing greater amounts of IL-4 and IL-13.[47]

Vaccine developments against *C. neoformans*

C. neoformans is a ubiquitous fungal pathogen mainly infecting immunocompromised individuals.[48] The first described vaccine to confer antibody-mediated protection against a systemic mycosis in an animal model was a conjugate vaccine of GXM, a major component of the cryptococcal capsule, coupled to tetanus toxoid. The vaccine was given to mice with monophosphoryl lipid A as an adjuvant, eliciting high levels of IgA and IgG against the capsular GXM. This immunization conferred 70%–80% protection against an intravenous moderate challenge with *C. neoformans*. The authors also suggested that IGs derived from high-tittered antiserum induced by this vaccine may be used as an immunotherapy for the management of cryptococcosis in patients with AIDS.[49] Nonetheless, the efficacy of the antibodies elicited against GXM-tetanus toxoid relies on the integrity of the whole immune system, since such efficacy seems to depend on T-cell function and its related cytokines,[50,51] the antibody isotype[52,53] and, more remarkably, the host genetics.[54] Likewise, using P13, a mimotope of GXM[55] linked to either tetanus toxoid or BSA that was preadsorbed to the aluminum hydroxide adjuvant Alhydrogel also induced a protective response against the fungus, prolonging survival

when compared to controls after administration of a lethal challenge[56] or after the establishment of a chronic infection, though the degree of protection was dependent on the carrier protein, route of infection, and also the genetic background of the host.[57]

On the contrary, alkaline extracts from mutant cryptococcal strains lacking capsule or chitosan were packaged into glucan particles, obtained from purified *S. cerevisiae* cell walls. These preparations were used for subcutaneous vaccination, providing significant protection against pulmonary infection with highly virulent strains of *C. neoformans* and *Cryptococcus gattii*.[58] Cytosolic proteins of the pathogen have also been used as an antigen. Khan et al described that poly-lactide co-glycolide microspheres encapsulated with cytosolic proteins further co-encapsulated into fibrin cross-linked plasma beads prompted an effective immune response by activating both CD4^{+} and CD8^{+} T cells, favoring IFN-γ- and IL-2-producing cells. Vaccination with this system also induced an IgG response, effectively cleared the fungal burden in vital organs, and increased the survival rate of immunized mice.[59]

An interesting approach to the development of strategies to confer immunity to fungal infections is the one explored by Wormley et al.[60] In this study, the authors genetically engineered a strain of C. neoformans to produce IFN-γ, and after the administration of this modified strain, mice were able to resolve the primary infection mainly by stimulation of local Th1-type anti-cryptococcal cell-mediated immune response and demonstrated full protection against a secondary pulmonary challenge with a pathogenic *C. neoformans* strain.[60] More recently, the same group explored the effect of this vaccination in mice with defective T-cell-mediated immune response. This was achieved by inactivating CD4^{+} or CD8^{+} T cells with anti-CD4^{+} or anti-CD8^{+} antibodies, respectively, prior to infection with the IFN-γ-producing *C. neoformans* strain. They found that the absence of CD4^{+} or CD8^{+} T cells, but not both subsets, resulted in survival to an acute pulmonary infection with the engineered strain and a subsequent second challenge with wild-type *C. neoformans* strain.[61]

Considering that antibodies stimulated by vaccination could defend against fungal infections, some investigations have been directed toward the generation of therapeutic MAbs. For example, it has been demonstrated not only that MAbs against melanin reduced the growth rate of in vitro melanized *C. neoformans* cells but also that passive immunization using these MAbs was able to extend the survival of mice infected with this fungus, suggesting the protection

of the MAbs to melanized *C. neoformans* could alter the properties of the cell wall melanin, thus interfering with cell replication.[62] Similarly, MAbs binding to *C. neoformans* capsule directly modified gene expression, and these effects correlated with antibodies binding to different locations of the capsule, ranging from downregulation of genes encoding for protein translation to differences in the pattern of phosphorylated proteins and changes in lipid metabolism that resulted in increased susceptibility to amphotericin B.[63] Casadevall et al developed 18B7, a murine MAb, against *C. neoformans* polysaccharide that showed interaction with all serotypes of the fungus, activation of the complement system, and promotion of phagocytosis.[64] More effective results have been accomplished by using this antibody conjugated to [213]Bi, a therapeutic radioisotope, against cryptococcosis in mice.[65]

Vaccine developments against *P. brasiliensis*

P. brasiliensis is a dimorphic fungal pathogen causing infection by inhalation of fungal conidia. Paracoccidioidomycosis is the most prevalent subcutaneous mycosis in Latin American countries, especially in Brazil.[66] Puccia et al reported that gp43 is a specific antigenic and exocellular component of *P. brasiliensis* found in culture filtrates[67] and ever since has been systematically studied as a potential vaccine. The T-cell epitope of gp43 is a 15-mer peptide (P10), and immunization of mice with both gp43 and P10 directed to strong protection against intratracheal challenge by virulent *P. brasiliensis*. The cellular immune response in mice to gp43 involves CD4^{+} Th1 cells producing IFN-γ and IL-2, unlike P10 in which the protective effect is mainly due to an IFN-γ-mediated cellular immune response and does not induce a humoral response.[68]

Since management of paracoccidioidomycosis often includes extended periods of chemotherapy, a combination of antifungal drugs and immunization with P10 were used together attempting to improve the treatment of the disease and to avoid relapses. This combination, in general, increased the levels of IFN-γ and IL-12 as proved to be a successful additive protective effect in mice after intratracheal challenge,[69] and similar results were obtained with this combined drug/P10 treatment in anergic mice[70] or using a DNA-based vaccination strategy with the gp43 gene.[71]

A few other antigenic molecules of *P. brasiliensis* have also been investigated, such as paracoccin and the Pb27 protein. Paracoccin, a dual-function protein having lectin properties as well as N-acetylglucosaminidase activities, is able to stimulate murine peritoneal macrophages to produce TNFα and nitric oxide and also induce Th1 immunity in mice

when injected prior to fungal challenge.[72] Immunization with the Pb27 protein, in addition to its protective effect, also proved to prevent pulmonary fibrosis in mice.[73]

Vaccine developments against *Sporothrix* spp.

Species belonging to the genus *Sporothrix* can be considered worldwide distributed, but endemic areas of the caused disease, sporotrichosis, are found in Latin America and Asia.[74] *S. brasiliensis*, *S. schenckii,* and *Sporothrix globosa* can cause lymphocutaneous or systemic fungal infections in healthy and immunocompromised hosts.[75,76] *S. brasiliensis* has been recognized as the most virulent strain and represents a threat to humans due to the massive zoonotic transmission in Brazil.[77]

The cell wall of *Sporothrix* represents a suitable source of antigens, and one is a glycoprotein of 70 kDa (Gp70) found in *S. schenckii* and *S. brasiliensis.*[75,78] Gp70 has been shown to be the immunodominant molecule of the *Sporothrix* cell wall.[75] Mice infected with *Sporothrix* are able to produce specific antibodies, such as IgG1 and IgG3 against Gp70, indicating its possible participation in controlling the infection.[79] Nascimento et al generated a MAb against Gp70, MAb P6E7, of the IgG1 isotype.[78] Yeast cells opsonized with MAb P6E7 had an increased phagocytic index and a reduction in the number of colony forming units in liver and spleen. Also, MAb P6E7 was able to inhibit the interaction of yeasts with the subendothelial matrix, given that this antigen is a putative adhesin for fibronectin and laminarin.[80,81] In addition, when the MAb was injected after infection, high levels of IFN-γ were detected, indicating that treatment with P6E7 may induce a protective cell-mediated response.[79] The passive immunization demonstrated to be protective before, during, and after infection, and even in mice infected with highly virulent strains.[82] Passive immunization with antibodies anti-Gp70 provided protection against sporotrichosis but was not able to induce the generation of long-lasting memory. These antibodies can be used as a treatment option, especially for immunocompromised patients, but not as stimulators of an active immunity.[76]

Portuondo et al[83] developed two vaccine formulation by using glycoproteins from the cell wall of *Sporothrix*, with different concentrations of proteins (AH-CWP100 and AH-CWP10). Sera from both AH-CWP100- and AH-CWP10-immunized mice enhanced yeast phagocytosis and inhibited its adhesion to fibroblast. In addition, IgG1 and IgG2 antibodies were induced by immunization with AH-CWP100, and passive transference of this serum was able to provide in vitro protection. Also, there was an increased

ex vivo release of IL-12, IFN-γ, IL-4, and IL-17, suggesting a balance of Th1, Th2, and Th17 responses.[83] Later, a PGA-CWP100 vaccine induced higher levels of IgG2, higher phagocytic index of yeast, but similar fungal load reduction, when compared to the AH-CWP100. The authors proposed that several mechanisms are involved in the protection given by the vaccine formulations that might depend on the adjuvant.[84] Most recently, Chen et al identified three epitopes from the Gp70 sequence and choose one (kpvqhalltplgldr peptide) to express it on a phage. They observed that immunization with the recombinant phage displaying the peptide could produce antibodies that bind to Gp70, which indicates that the peptide has a similar response to that of the whole Gp70, in the treatment of sporotrichosis. The recombinant phage-induced antibody production protected mice infected with *S. globosa*, showing a reduction of the fungal load, a decrease in the number of inflammatory cells and a higher survival rate (80%) of animals.[85] This could be a potential vaccine candidate against *Sporothrix*, given that the presence of Gp70 has been confirmed in *S. schenckii*, *S. brasiliensis,* and *S. globosa*.

Vaccine developments against other fungal pathogens

BAD1 is a surface adhesin and an immunodominant antigen of *Blastomyces dermatitidis* that generates cell-mediated and humoral responses.[86] Recombinant BAD1 immunization is able to prolong survival of mice to lethal pulmonary blastomycosis, but most mice succumb to infection, even when IL-12 is used as an adjuvant. Wuthrich et al[87] tested a recombinant, live, attenuated vaccine against *B. dermatitidis,* the BAD1 null mutant. The mutant is unable to establish a lethal pulmonary infection since mice infected with it could control the multiplication of yeast and generated acquired immunity. Immunized mice with viable yeasts of the mutant showed a decreased fungal burden in lungs and developed a delayed-type hypersensitivity and polarized type 1 cytokine response, linked with resistance.[87] This protection was correlated with the production of IFN-γ and T-cell activation and proliferation.[86]

In the case of *Histoplasma capsulatum*, a 62 kDa protein (termed HIS-62) was isolated from yeast cells. It was determined that this antigen is a target of the cellular response and that is able to confer protection against the mycosis. In mice infected intravenously with yeast cells, immunization with the protein induced a high proportion of monoclonal population of T cells and stimulated a protective immune response.[88] Also, immunization stimulates the production of

IL-10, IL-12, and IFN-γ.[86] Later, the recombinant HIS-61 was expressed and tested for immunological activity. Vaccination with this protein caused an increase in the recognition of the yeast by the lymphocytes and a higher survival rate, providing protection against a lethal intranasal challenge with yeast in a mouse model.[89]

Once an infection caused by *Coccidioides* is resolved, memory immune cells confer lifelong immunity against a re-infection. Several antigens of the fungus have been identified and tested to see whether they might induce a similar protection than that of the natural infection.[90] Two antigens have recently been tested, a proline-rich cell wall protein, Ag2/PRA, and a secreted protein, *Coccidioides*-specific antigen (CSA). Protection has been reported for the recombinant protein Ag2/PRA and its truncations, but the CSA has not been implicated in a protective response yet. However, Shubitz et al[91] developed a vaccine based on a chimeric fusion protein composed of Ag2/PRA$_{1-106}$ co-expressed with CSA and tested it in a mouse intranasal challenge model. They observed greater survival of mice when immunized with the chimeric protein, compared to either antigen alone.[91]

Passive immunotherapy

The use of antibodies to neutralize an infection without inducing an active memory immune response by the host is another way to combat pathogens. Mycograb® a human recombinant antibody against Hsp90, a chaperone essential for cell viability in *Candida* spp., shows antifungal activity and synergy with amphotericin B. *Aspergillus* spp. and *C. neoformans* also possess Hsp90; therefore, this antibody could also be used against these pathogens.[92] The 18B7, a mouse MAb directed against *C. neoformans* capsular polysaccharide component, GXM could be used as anti-capsular antibody therapy, promoting opsonophagocytosis of the fungi or clearance GXM.[93] *H. capsulatum* is another pathogenic fungus that expresses in the cell surface an antigen identified as a histone H2B-like protein, and the administration of a MAb directed against it decreased the fungal burden and pulmonary inflammation and increased the yeast phagocytosis by J774.16 cells through a CR3-dependent process.[94] MAbs both IgM(B6.1) and IgG3(C3.1) directed against a β1,2-mannotriose epitope of *C. albicans* cell surface have a protective role in a murine model of candidiasis.[53] The killer toxin, which interacts with β-glucans, is another option to combat fungal infections through passive immunotherapy.[95] The anti-idiotypic killer toxin antibodies were produced by immunizing animals with killer toxin.[96] These antibodies decreased vaginal candidiasis and *Pneumocystis carinii* pneumonia.[95]

Concluding remarks

The increased frequency of multidrug resistance in fungal isolates and the emergence of new species naturally resistant to the current chemotherapeutic alternatives, such as *Candida auris*,[97] have stressed the need for alternatives to control fungal pathogens. The examples given in this review paper underscore the effort in this line, and currently, there are some antigens/antibodies with the potential to confer protection against fungal pathogens tested in clinical trials.[23] We have to take this information carefully, as fungal organisms have several strategies to evade immune recognition,[1] and there is a risk of failure in the immunization against the pathogen, as in the case of *C. albicans*.[23] Multivalent antigenic preparations might represent an alternative to divert this potential risk, and this is a challenge of high priority to address. Furthermore, the study of universal vaccines, like WGA-Fc, a chimera that binds chitin and has antifungal properties,[98] may open new alternatives to generating a vaccine that is able to protect against not only one but several fungal pathogens. Finally, the combination of antifungal drugs and immunostimulants, like fungal antigens, is another promising area to develop new therapeutic schemes that need further development.

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Disclosure

The authors reports no conflicts of interest in this work.

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