



Vaccine Development to Systemic Mycoses by Thermally Dimorphic Fungi

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Abstract

Purpose of Review Pathogenic thermal-dimorphic fungi are endemic in certain regions and can cause from subclinical respiratory infections to systemic mycoses. These pathogens are associated with high rates of mortality and high morbidity, infecting thousands of people each year. In addition, the toxicity and high costs of treatment of systemic mycoses are great public health concerns. In the present review, we address recent studies that refer to the development of vaccines against systemic mycoses by thermally dimorphic fungi.

Recent Findings Members of the genus *Paracoccidioides*, *Histoplasma*, *Coccidioides*, and *Blastomyces* are thermal-dimorphic fungi, and the difficulty in obtaining new and selective antifungal therapies led to the increase of research involving development of new options of immune therapy. Immunotherapeutic strategies and new vaccines have been focused on protecting populations at risk and assisting in antifungal treatment, reducing the time of therapy and toxicity. Peptides, purified antigens, DNA therapy, dendritic cells, in addition to the use of attenuated yeast cells and monoclonal antibodies, have been explored as potential vaccines.

Summary In recent years, despite advances in the search for new antifungal therapies with a focus on the development of prophylactic and/or therapeutic vaccines, few prototypes of successful treatment have emerged from clinical trials. It is clear, however, that all information from these studies, concerning the pathogen-host relationship and the understanding of the immune response to these microorganisms, are indispensable for the development of new treatment options aiming at reducing the morbidity and mortality of populations at risk for these infections.

Keywords Thermally dimorphic fungi · Systemic mycoses · Antifungal vaccines · Fungal infections

Introduction

The increased number of fungal infections in recent years is directly related to the immunocompromised populations, with a serious impact on the health care systems [1, 2]. In the past, HIV-positive individuals were the main risk group of fungal

infections. Nowadays, patients undergoing chemotherapy as in cancer, organ transplantation, premature neonates, and individuals in intensive care units, are also risk groups of invasive mycoses [3].

Pathogenic thermal-dimorphic fungi are agents of subclinical respiratory infections to systemic mycoses, being endemic in certain regions. Among these fungi, are members of genera *Paracoccidioides*, *Histoplasma*, *Coccidioides*, and *Blastomyces*. These microorganisms are present in the soil, plant remains, and/or animal excreta. The infection occurs through the inhalation of propagules found in the environment and the fungal cells can spread to the entire body. These microorganisms are responsible for high rates of mortality and high morbidity in endemic areas, by infecting thousands of people each year. Epidemiological data are not, however, accurate, as these mycoses are underreported (Reviewed in [4]).

Paracoccidioides spp. form a complex of species that are responsible for paracoccidioidomycosis (PCM), with the main

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etiological agents being *P. brasiliensis* and *P. lutzii*. Recently, three other species have been suggested to be included in this genus: *P. restrepiensis*, *P. americana*, and *P. venezuelensis* [5]. PCM is a systemic mycosis throughout Latin America, with great incidence in Brazil, Venezuela, Colombia, and Argentina [6]. According to the literature, more than 80% of cases of PCM are reported in Brazil, where the main infected population comprises rural workers [7, 8].

Histoplasmosis is an endemic mycosis, caused by *Histoplasma capsulatum*, frequent in the USA, Mexico, and South America, with case reports in China and India [9, 10]. It is an opportunistic mycosis, and HIV positive individuals are those mainly affected by this fungus in Latin America [11]. Infection occurs by inhalation of microconidia from the environment. In the absence of some form of immunosuppression, the infection is usually contained [12].

Coccidioidomycosis is a fungal infection caused by dimorphic, soil-dwelling *Coccidioides immitis* and *C. posadasii*, which are endemic in regions of Arizona, California, New Mexico, Nevada, Utah, Washington, Texas, and Mexico. There are also cases in some areas in Venezuela, Brazil, Argentina, and Paraguay. According to the literature, cases of *Coccidioides* spp. have been reported in increasing numbers in recent years probably due to changes in environmental conditions, human activities in endemic areas, immunosuppressed individuals, and the development of more accurate diagnostic tests (reviewed in [13]. The infection starts by inhalation causing a broad spectrum of disease, including mild febrile illness, severe lung infection and disseminated disease [14].

Blastomycosis can be caused by the agents *Blastomyces dermatitidis* and *B. gilchristii*, with high incidence in Canada and Eastern USA. Clinical manifestations are diverse, ranging from asymptomatic infection to extrapulmonary spread, reaching almost all organs of the body [15].

Currently, antifungal treatment options are limited, especially in developing countries. Although effective in most cases, the treatment of fungal infections is toxic, expensive, and must often be extended to a couple years [16]. In addition, the emergence of resistant strains and increase of relapsing disease are common clinical concerns [17].

The choice of antifungal drugs and treatment time in systemic mycoses depend on the severity of the infection [18]. Amphotericin B (AmB) is indicated to treat severe cases whereas azoles are preferred for the moderate ones [19]. In the case of PCM, the use of trimethoprim-sulfamethoxazole is also acceptable, presenting relative success. Echinocandins are a class of antifungal agents that cannot be used to treat mycoses caused by dimorphic fungi, since they have a natural resistance to these drugs [4, 20].

In order to achieve effective antifungal treatment against dimorphic fungi, therapy generally has to be administered for long periods, and even then, the infection may not be

eliminated. Treatment in less severe cases can last up to 1 year, but in the cases of disseminated infection or in cases of disease in immunocompromised patients, therapy must be held beyond this period [4].

In recent years, concern about the increased incidence of serious infections caused by fungi, together with a difficult management therapy, stimulated the search for new therapeutic options [21]. Thus, the use of immunotherapeutic strategies has gained interest and the development of vaccines that can protect populations at risk or assist in antifungal treatment, reducing the time of therapy and drug toxicity, is a goal that many studies aim to achieve.

This review addresses some studies on the development of vaccines to systemic mycoses, including PCM, histoplasmosis, coccidioidomycosis, and blastomycosis.

Paracoccidioidomycosis

Aiming at a vaccine against *P. brasiliensis*, yeast cells of Pb18 strain were gamma irradiated with doses between 0.5 and 8.0 kGy. Fungal cells after gamma radiation became less virulent, and their proliferation was inhibited. Their metabolic activity was still present and the antigenic profile was maintained [22]. When using gamma irradiation at 6.5 kGy, alterations in the yeast morphology were reversible after 48 h. Electron microscopy showed nuclear alterations without damage in both plasma membrane and cell wall [23]. Looking for a diagnostic and protective antigen in *P. brasiliensis*, the 43-kDa glycoprotein (gp43) was identified [24]. The epitopes in gp43 that elicited a specific antibody response in patients with PCM are peptidic in nature therefore reacted with the deglycosylated molecule [25].

The gp43 antigen is also able to elicit positive skin tests in guinea pigs and in humans implying the presence of an immunodominant epitope to a CD4⁺ T cellular immune response [26, 27]. This epitope was mapped by Taborda et al. [28] to a specific sequence of gp43, QTLIAIHTLAIRYAN, denominated P10. This peptide has a hexapeptide core (HTLAIR), as the essential domain of the IFN- γ -dependent Th1 epitope recognized by H-2^b isogenic mice. Previous immunization with P10 or gp43 in the presence of complete Freund's adjuvant (CFA), protected mice intratracheally infected with *P. brasiliensis*, significantly reducing the fungal burden in the lungs, liver and spleen [28]. In a study using TEPITOPE algorithm, the P10 emerged as a good candidate for a human vaccine, since this peptide was found to be promiscuously presented by MHC class II molecules from mice and humans [29, 30]. In recent years, several studies on the different ways of peptide delivery and other properties of P10, have contributed to validate this peptide as a potential candidate for a human vaccine [20, 31–33].

Marques et al. [34], aiming at improving the treatment of PCM mainly to avoid relapses of the disease, carried out a study using P10 immunization combined with antifungal drug treatment. Using two protocols, the use of P10 in combination with antifungal drugs showed an additive protective effect in the experimental model using BALB/c mice. Th1 and Th2 cytokines were present in successfully treated mice and that the combined treatment with P10 immunization reduced the CFUs and maintained preserved lung alveolar structure. In addition, the combined treatment prevented the spread of the fungus to other organs. Subsequently, in order to mimic the anergic state of some patients with acute and subacute forms, the effects of combined treatment of trimethoprim and sulfamethoxazole or itraconazole, with P10 immunization in immunocompromised mice, were investigated. Anergic mice infected with *P. brasiliensis* showed high fungal loads in the liver, spleen and brain after 45 days of infection, in addition to high dosing of IL-4 and IL-10 and low levels of IL-12 and IFN- γ . This pattern was reversed by P10 immunization and not with antifungal drugs. With the combined therapy, a high Th-1 type immune conversion was seen. In addition, the immunization with P10 significantly reduced the fungal burden in lung, spleen and liver of anergic mice [35].

The protective efficacy of IFN- γ in the PCM murine model was demonstrated, as mice deficient in IFN- γ or IFN- γ receptor evinced 100% mortality after infection with a virulent isolate of *P. brasiliensis* (reviewed in [36]).

Amaral et al. [37] carried out a study using three strategies of combined therapy, in which animals infected with *P. brasiliensis* received daily, sulfamethoxazole/trimethoprim alone or in conjunction with P10 and Freund's adjuvant or P10 in poly-lactic acid-glycolic acid (PLGA) nanoparticles. In addition to the effectiveness of combined treatment with P10 in PLGA, the required amount of P10 to obtain protective results lowered significantly. P10 incorporated into PLGA was protective for prolonged periods compared with the combined treatment using P10 emulsified in Freund's adjuvant. Using mice treated with dexamethasone, immunization with P10 has been shown to efficiently modulate the immune response in immunosuppressed hosts, increasing animal survival, reducing lung fungal load, and also the pulmonary fibrosis in mice immunized with the peptide [38]. The use of gene therapy has also been described as being an alternative to PCM, being able to induce memory cells [39]. Tested in intratracheally infected mice, the pcDNA3 expression vector encoding P10 induced a significant reduction of fungal load in the lung. Additionally, when also using the plasmid encoding mouse IL-12 a more significant activity in the elimination of the fungus [40] was obtained.

DNA vaccine based on HSP65 from *Mycobacterium leprae* against PCM was able to induce protection against infection by *P. brasiliensis* through the production of IFN- γ and IL-12 [41]. Subsequently, controlled release systems were

used to evaluate the DNAhsp65 vaccine and the effects of this strategy on pulmonary PCM treatment. Mice that received DNAhsp65 vaccine released by different nanoparticles showed significant decrease in CFUs when compared with the treatment using only DNAhsp65. In addition, this effect was obtained using about four times less DNAhsp65, demonstrating the efficiency of the controlled release system. The protection of animals is related to increased production of protective type Th1 cytokines, IFN- γ and IL-12 [42].

P. brasiliensis affects healthy individuals and, generally, the defense against this infection in the lungs requires a good innate and adaptive immune response. Dendritic cells (DCs) are efficient inducers of T lymphocyte immune responses against *Paracoccidioides* antigens [43–45]. The administration of gp43-pulsed DCs to mice resulted in increased production of IL-2 and IFN- γ by CD4⁺ T cells isolated from regional lymph nodes [46]. Subcutaneous or intravenous administration of DCs pulsed with P10 has subsequently been shown to confer protective immunity against infection by *P. brasiliensis* in the murine model. Mice receiving DCs pulsed with P10 showed a mixed cytokine response pattern with predominance of Th1 activation and significant reduction of fungal burden compared with control animals [43, 45, 47].

In a study using rPb40 and rPb27 recombinant proteins, Fernandes et al. [48] analyzed the therapeutic activity of these proteins in combination with fluconazole (FLZ). A significant therapeutic effect was observed, to the point of no recovery of CFU from lungs, livers, and spleens of BALB/c mice, immunized after 4 months of *P. brasiliensis* yeast infection. Animals submitted to the combination therapy showed low IL-4 and TGF- β and increase in TNF- α after 70 days of infection and high levels of IFN- γ after 120 days of infection.

Investigators have attributed to TNF- α the control of dissemination and growth of the fungus, assisting in the regulation of inflammatory responses in PCM [49]. TNF- α acts by attracting and activating effector cells and is extremely important in the granulomatous inflammatory reaction [50, 51]. In contrast, IL-4 can inhibit the production of IFN- γ and hinder the activation of macrophages (reviewed in [48]).

Holanda et al. [52] constructed two types of vaccines (P10/His) 6-purified protein (rPbT) and recombinant P10/human type 5 adenoviral vector (rAdPbT)), based on chimeric virus-like particles (VLPs), containing the main CD4⁺ T cell-specific epitope from *P. brasiliensis* (P10) within the C-terminal portion of HBcAg. As a result, the authors first proved that the recombinant VLP protein carrying a specific CD8⁺ T cell epitope from murine cytomegalovirus (rCMV), did not protect the infected animals, demonstrating that the use of chimera is valid for the evaluation of P10-based vaccine efficacy through the specificity of the CD4⁺ T cell response. Mice were immunized with rPbT and rAdPbT, and the Pb18 strain was used for infection of animals. High levels of protective Th-1 cytokines were formed, inducing a CD4⁺ T

response. In addition, when rAdPbT was pre-inoculated it prevented the spread of PCM in the infected mice.

Recently, the recombinant single-chain variable fragments (scFv), that mimic Ab2- β 7B12 mAb against gp43 of *P. brasiliensis*, incorporated into PGLA nanoparticles, modulated the production of cytokines, which protected against PCM. The cytokine profile in the lung of animals receiving scFv-nanoparticles showed increased IFN- γ and IL-12 and low IL-4 and IL-10 production, resulting in decreased fungal burden in treated mice. The authors suggest that the treatment modulated the humoral immune response with production of IgG2a and IgG1. Despite the positive data demonstrated, the results obtained in the prophylactic treatment need to be further developed [53].

The use of monoclonal antibodies (mAb) was also tested against PCM caused by *P. brasiliensis*, where anti-gp43 and anti-gp70 demonstrated efficacy in the contention of the experimental infection.

In addition to gp43, gp70 is also recognized in the serum of most PCM patients. This glycoprotein inhibited the action of peritoneal macrophages, suggesting a mechanism of action similar to that of gp43. With this in mind, a study was carried out where mAb were produced against gp70. When studying the activity of anti-gp70 IgG1 mAb by passive immunization of mice infected with *P. brasiliensis*, significant reduction in the recovery of CFUs from the lungs of the animals was shown when the C5F11 and B7D6 mAb were combined [54].

The biological function of *P. brasiliensis* 75 kDa protein was verified using IgM and IgG mAb. In vitro assays showed that these mAb were able to inhibit fungal growth. After yeast opsonization, the phagocytosis by murine peritoneal macrophages was stimulated. In addition, the fungal burden decreased significantly when passive immunization with mAb was performed [55].

Buissa-Filho et al. [56] analyzed the 19G, 10D, 32H, and 17D (IgG2a) and 21F and 3E (IgG2b) mAb against gp43 by passive immunization of mice infected with *P. brasiliensis*. All mAb tested increased the phagocytosis rate in vitro, but the 3E showed a more significant result. Through passive administration of the antibodies protective and non-protective mAb were identified and the protective ones reduced the fungal burden in the lungs. It has also been shown that the epitope for 3E mAb, NHVRIPIGYWAV, is homologous with β -1,3 glucanase sequences from other fungi. Since gp43 can be found on the cell surface of *P. brasiliensis* [57], the opsonizing effect of 3E mAb may involve the recognition of this peptide sequence on the cell wall of the fungus. Although PCM protection is associated with a potent cellular response, this work demonstrates that protective anti-gp43 antibodies can also assist in the infection treatment in mice.

P. lutzii infections did not respond to serological tests using gp43. Thomaz et al. [58] analyzed the effects on *P. lutzii* experimental PCM, of two mAb generated to the heat shock

protein from *H. capsulatum* (Hsp60), 7B6 (IgG2b), and 4E12 (IgG2a), since there is homology among the Hsp families from dimorphic fungi. The authors observed that passive transfer of 7B6 and 4E12 mAb to Hsp60 decreased the fungal load in the lungs of infected mice. In murine histoplasmosis, IgG2a mAb is associated with a Th1 response whereas mAb IgG2b induced a Th2 response, exacerbating the disease. In murine PCM caused by *P. lutzii*, both mAb were protective suggesting the activation of a Th1 response. In addition, the authors argue that the effects caused by mAb on phagocytosis are due to the significant increase in IFN- γ production, as documented in studies with *P. brasiliensis*.

The vaccine candidates against PCM are summarized in Table 1, and the main monoclonal antibodies tested in Table 2.

Histoplasmosis

In the last years, several vaccine candidates against *H. capsulatum* have been studied such as: cell wall and membrane extracts from *H. capsulatum* yeast cells [59]; the 62-kDa protein, named HIS-62 [60]; rH antigen [63, 78]; native and recombinant Heat Shock Protein 60 [62, 79]; extracts containing cell-free antigens from *H. capsulatum* yeast [64]; and alkaline extract of *H. capsulatum* packaged in glucan particles [65].

In 1991, Gomez et al. [59] did experiments to analyze T cell antigens from *H. capsulatum* yeast cells. An extract of cell wall and membranes from *H. capsulatum* yeast cells was prepared and tested to evaluate the antigenicity and immunogenicity of the extract. The preparation induced a cell-mediated immune response both in vivo and in vitro [59].

In another work, a 62-kilodalton antigen, HIS-62, from the cell wall and cell membrane of *H. capsulatum* yeast cells, was used to prophylactically vaccinate mice and a protective effect was observed against a lethal intravenous challenge with yeast cells, during a 28-day observation period [60].

Gomez et al. [61] after demonstrating the protective effect of HIS-62, a member of HSP-60 family, cloned the gene encoding this antigen and studied the immunological activity of the recombinant antigen (rHIS-62). They observed that BALB/c mice vaccinated with the recombinant protein were protected against a lethal intranasal challenge with yeast cells and also observed that splenocytes from mice immunized with *H. capsulatum* showed a strong response to rHIS-62.

The H antigen is an important antigen used to discriminate active and remote infection. Deepe and Gibbons [63] have demonstrated that the recombinant H antigen (rH) is able to protect C57BL/6 mice immunized with the recombinant protein and challenged with lethal and sublethal inoculum (intranasally) of yeasts of *H. capsulatum*. Increased production of IFN- γ , and GM-CSF was observed in spleen cells from

Table 1 Vaccine candidates tested against systemic mycoses by thermally dimorphic fungi

Mycosis	Candidate	Types of response/immunity	Reference work
Paracoccidioidomycosis	P10	Th1	[28, 38]
	P10 combined with antifungal drugs	Th1	[34, 35]
	P10 incorporated into PLGA with antifungal drugs	Th1	[37]
	P10 DNA vaccine	Th1	[40]
	DNA vaccine based on HSP65 from <i>Mycobacterium leprae</i>	Th1	[41, 42]
	P10 pulsed DCs	Predominance of Th1 activation	[43, 45]
	rPb40 and rPb27	Th1	[48]
	rPbT and rAdPbT	Th1/CD4 ⁺ T cell response	[52]
Histoplasmosis	scFv into PGLA	Th1 and IgG2a/IgG1 production	[53]
	Extract of cell wall and membranes	T cell proliferation	[59]
	HIS-62	T cell proliferation	[60]
	rHIS-62	T cell proliferation	[61]
	rHsp60	T cell V β 8.1/82 ⁺	[62]
	Recombinant H antigen	Increased production of IFN- γ and GM-CSF in spleen cells	[63]
	Extract containing cell-free antigens	High production of IFN- γ (spleen cells)	[64]
	Alkaline extract of <i>H. capsulatum</i> packaged in glucan particles	Th1 and Th17	[65]
Blastomycosis	BAD1	Th1 and Th17	[66–69]
Coccidioidomycosis	PRA	Th1	[70]
	Δ T (cts2/ard1/cts3 Δ)	Th1 and Th17	[71, 72]
	Pep1	Th1	[73]
	rEBV (Pep1-P1, Pep2-P2, Amn1-P10, Amn11-P11 and Plb-P6)	Th1 and Th17	[74]

mice immunized with rH and stimulated in vitro with 25 μ g of antigen/ml for 24 h.

Scheckelhoff and Deepe [62] have shown that immunization with recombinant heat shock protein 60 (rHsp60) from *H. capsulatum* or fragment 3 (F3), a specific region of the protein, conferred protection in mice challenged with the fungus. The experiments demonstrated that rHsp60 causes the expansion of murine T cells that predominantly express the V β 8.1/82 region TCR and that the elimination of V β 8.1/82⁺ cells causes loss of protection efficacy by rHsp60.

Sá-Nunes et al. [64] standardized the production of extract containing cell-free antigens (CFAGs) and observed in vitro that the spleen cells from mice immunized and stimulated with these antigens produced high amounts of IFN- γ and that immunization with CFAGs was able to induce protective immunity, reducing fungal burden, blocking the dissemination of *H. capsulatum* and protecting infected mice from lethal inoculum.

In 2018, an alkaline extract of *H. capsulatum* packaged in glucan particles was evaluated in a murine model. The results

Table 2 Main monoclonal antibody tested against paracoccidioidomycosis, histoplasmosis, blastomycosis, and coccidioidomycosis

Mycosis	mAb	Target	Reference
Paracoccidioidomycosis <i>P. brasiliensis</i> and <i>P. lutzii</i>	C5F11 and B7D6 (IgG1)	Gp70	[54]
	1G6 and 5E7C (IgM and IgG)	75 kDa	[55]
	3E (IgG2b)	gp43	[56]
	7B6 and 4E12 (IgG2b and IgG2a)	Hsp60	[58]
Histoplasmosis	IgM	Histone H2B-like protein	[75]
	11D1, 6B7, 4E12, 12D3, 13B7 IgG1, and IgG2a	Hsp60	[76]
Blastomycosis	BD6-BC4, DD5-AD3, DD5-AD9, DD5-AD11, DD5-AE5, DD5-CB4, CA5-AA3, and CA5-BC12	120-kDa (Wi-1)	[77]

demonstrated that the alkaline extract in glucan particles was able to protect mice against lethal and sublethal challenges with *H. capsulatum* yeast cells. Vaccine with alkaline extract induced Th1 and Th17 response in lungs and draining lymph nodes and a small population of IL-17⁺ IFN- γ CD4⁺ was detected [65].

In histoplasmosis, it has been observed that B cells are effective in the immune response against the fungus [80]. In 2006, a study showed that depletion of CD4⁺ and CD8⁺ T cells in B cells^{-/-} mice, the fungal burden increased considerably when compared with the wild type [80]. In other studies, the efficacy of mAb therapies has been demonstrated [75, 76]. Nosanchuk et al. [75] performed passive administration in mice of mAb to a surface histone-like protein of *H. capsulatum* (IgM) and observed the reduction of fungal burden in organs and prolonged survival when mice were challenged with lethal inoculum of *H. capsulatum*. Increased IL-4, IL-6, and IFN- γ in the lungs of treated mice were associated to increased protection. In another study with IgG1 and IgG2a mAb to *H. capsulatum* HSP60, prolonged survival of mice was obtained when they were challenged with a lethal inoculum of 1.25×10^7 *H. capsulatum* yeast cells compared with control mice receiving either irrelevant mAb or PBS. An IG2b mAb, however, was not protective. In conclusion, IgG1 and IgG2a mAb to *H. capsulatum* HSP60 can modify the pathogenesis of murine histoplasmosis [76].

All of the information about the vaccine candidates against histoplasmosis and the main monoclonal antibodies tested is summarized in Tables 1 and 2, respectively.

Blastomycosis

Blastomycosis can be classified as an acute subclinical infection evolving to chronic pneumonia. Although blastomycosis has the primary site in the lung, infection can become widespread, often in immunocompromised individuals, and reach the skin, bone, genitourinary system, and CNS [81].

For a protective immune response, CD4⁺ T cells play a major role in mediating resistance to fungal infection. CD4⁺ T cells confer resistance through secretion of T-helper (Th) 1 or Th17 cytokines such as IFN- γ , TNF- α , GM-CSF, and IL-17A, respectively, which activate neutrophils, macrophages, DCs and inflammatory monocytes for fungal clearance. Protective antibodies are produced [82, 83].

Blastomyces adhesin 1 (BAD1) is an avirulent strain created from disruption of the WI-1 gene [66, 67, 84]. Vaccination with BAD1 was able to elicit a protective response in mice vaccinated and infected with *B. dermatitidis*, increasing the frequency of CD4⁺ $\alpha\beta$ T cells and production of Th1 cytokines, mainly TNF- α , and to a lesser extent IFN- γ [85]. In the murine infection model performed with BAD1, it was shown that CD4⁺ T cells, apparently, are not needed for a

protective immunity to *B. dermatitidis* infection and that CD8⁺ T cells alone mediate and induce lasting and persistent resistance to the fungus. In *B. dermatitidis* infection in CD4⁺ T cell deficient mice, CD8⁺ T cells were able to produce proinflammatory cytokines (TNF- α , IFN- γ , and GM-CSF) linked to vaccine resistance and crucial in fungal control. Elimination of CD8⁺ T cells from BAD1 vaccinated (KO) mice during the vaccine expression phase greatly abolished their resistance [86]. The fact that CD4⁺ T cells are dispensable for the success of immunological vaccination against pulmonary mycosis (blastomycosis and histoplasmosis) is important, since the highest incidence of opportunistic fungal infections occurs in immunocompromised patients including patients with AIDS. Nanjappa et al. [87], using various experimental models of CD4⁺ T cell deficient mice vaccinated with BAD1 and infected with *B. dermatitidis*, proved that CD8⁺ T cells persist in number and function, even without the residual antigen, and that IFN- α producing CD8⁺ T cells express CXCR3 chemokines receptor, and its blocking prevents the influx of CD8⁺ T cells into the lungs.

The role of Th17 signaling is controversial in the immune response against fungal infection [88, 89]. To elucidate its role in the protection induced by vaccination with engineered, live, attenuated BAD1 vaccine, Th17 response and cytokine IL-17A were evaluated. The murine experimental model has shown that both response and cytokine may be engaged in vaccination to confer resistance against multiple dimorphic fungi. Neutralization of IL-17A using mAb and vaccination of IL-17^{-/-} mice showed that the cytokine is required for vaccine resistance, even in mice with normal Th1 cells, and that Myd88 adaptor protein is critical in inducing a Th17 response and vaccine immunity. The lack of it, therefore, impaired the induction of the Th17 antigen-specific Th1 response [68].

Studies with Dectin-1, a standard recognition receptor (PRRs) for fungi, involved in the innate Syk kinase signaling pattern [90, 91] and Card9 adaptor [92], critical in inducing the Th17 response to fungal infections, showed that the receptor is also dispensable in the development of BAD1 vaccine resistance and Th17 response induction [68]. This is in contrast to the vaccine-protective response induced against *H. capsulatum* and *C. posadasii* where Dectin-1 is required for the development of the Th17 immunogen response [93]. The differentiation of the Th17 immune response in blastomycosis is via Myd88, and Myd88^{-/-} (KO)-mice failed to control yeast vaccinations and were unable to recruit antigen-specific Th17 and Th1 cells to the lung to combat infection [68].

On the other hand, the Card9 adaptor protein is essential for the antifungal Th17 protective response in BAD1 vaccination in *B. dermatitidis* infection model [69]. Card9 (KO)-mice infected with *B. dermatitidis*, and unvaccinated, were more susceptible to infection [94]. Card9 is required for differentiation of Th17 cells, governing the expression of instrumental cytokines in the targeting of the Th17 and Th1 protective response.

Thus, the Dectin-2/FcR γ /Syk/Card9 signaling pattern is indispensable for the differentiation of Th17 and Th1 cells in resistance acquired infection by *B. dermatitidis* [69].

The BAD1 vaccine model also evaluated the use of IL-1 β cytokine as an adjuvant in the murine model of blastomycosis infection. IL-1 β is a proinflammatory cytokine that enhances the expansion and differentiation of CD4⁺ and CD8⁺ T cells [95, 96]. IL-1 β as an adjuvant in BAD1 vaccination owes its protective effect to the decreased CFU of mice vaccinated with BAD1 and infected with *B. dermatitidis*; to the frequency and number of IL-17 producing cells. The use of the cytokine in the vaccination helped to promote differentiation of Th17 antigen-specific and reduced the generation of Th1 and Th2 antigen-specific cells [97].

In search for a protective immune response against infection by *B. dermatitidis*, a 120-kDa surface protein was identified, named WI-1 [66]. It is an adhesin that binds the fungus to complement and an immunodominant antigen recognized by infected patients [98, 99]. The immunization with WI-1 elicits immune response in a murine model and enhances resistance to pulmonary infection, with increased IFN- γ and decreased CFU, while eliciting an IgG humoral response [77, 100]. Utilization of monoclonal antibodies to WI-1, in a study of five mAb (BD6-BC4, DD5-AD3, DD5-AD9, DD5-AD11, DD5-AE5, DD5-CB4, CA5-AA3, and CA5-BC12), no improvement in the immune response was observed against *B. dermatitidis*.

All of the information about the vaccine candidates tested against systemic mycoses and the main monoclonal antibodies tested against blastomycosis are summarized in Tables 1 and 2, respectively.

Coccidioidomycosis

Coccidioidomycosis, commonly known as San Joaquin Valley fever or Valley fever, is a fungal infection with high morbidity and potential mortality affecting persons in endemic areas [101]. Dimorphic etiologic agents, *Coccidioides immitis* and *Coccidioides posadasii*, are morphologically identical and their predicted proteins are more than 90% homologous [102]. The fungal mycelium can produce arthroconidia (spores) by hyphal segmentation in the soil, which are aerosolized and capable of causing infection by inhalation. The inhaled spores first convert to spherule initial forms, which grow in the lungs to multinucleate spherules [101, 103]. The vast majority of infections are mild and self-limited, usually leaving no residual damage greater than a pulmonary granuloma [104]. In contrast, a small percentage of infections do not resolve spontaneously, giving rise to chronic cavitary pulmonary infections or extrapulmonary infection foci, with high antibody titers. The higher these titers, the worse the prognosis of the infection [93].

Vaccine-induced immunity against coccidioidomycosis seems feasible because symptomatic second infections with *Coccidioides* spp. are extremely rare [105]. Successful vaccination requires a T cell-mediated immune response with both Th1 and Th17 playing a role in the vaccine-mediated protection [106]. There is no evidence that antibody response is protective in humans as there is no association between inherited or acquired immunoglobulin deficiencies and disseminated coccidioidomycosis, but in mouse models, there is conflicting evidence whether B cells are needed for vaccine-induced immunity [107, 108].

The proline-rich antigen (PRA) of *Coccidioides immitis* was studied as a vaccine candidate for coccidioidomycosis [70]. It is a glycopeptide component of 194-aa [109]. Using recombinant PRA (rAg2/PRA) conjugated with monophosphoryl lipid A (MPL) adjuvant in prophylactic immunization, a reduction in the fungal load of infected lungs was obtained, with the production of high levels of IFN- γ in splenocytes of immunized mice re-stimulated with rPRA [70].

An attenuated lineage of *Coccidioides posadasii* was also used as a vaccine candidate for coccidioidomycosis. The lineage used was deficient in chitinases involved in the stage of differentiation of the endospore in the parasitic life cycle. Studies with live and attenuated lineage (cts2/ard1/cts3 Δ strain) designated Δ T, showed that it could protect BALB/c mice susceptible to lethal infection with *C. posadasii*. Infected and non-vaccinated C57Bl/6 mice succumbed to infection 3 weeks after challenge, whereas another group vaccinated with the *C. posadasii* mutant had 100% survival 75 days post-challenge. The vaccine had fewer reatogenic effects than the previous formulation using formalin killed beads (FKS). The live and attenuated line used in the immunization tests elicited Th1 cytokines, with activation of CD4⁺ T cells and secretion of IFN- γ and IL-12p70 and marked decrease in the production of IL-6, a cytokine correlated with disseminated coccidioidomycosis [71].

The vaccine decreases proinflammatory cytokine levels (IL-1 β , IL-1 α , TNF- α , IL-6, and G-CSF) and also the number of neutrophils in the pulmonary infiltrate. The number of tissue and alveolar macrophages increased in the protected animals as also did that of CD4⁺ and CD8⁺ T cells in the pulmonary infiltrate of vaccinated mice. Chemo-attractants of macrophages, monocytes, and dendritic cells (CCL2/MCP-1 and CCL3/MPI-1 α) were also found in high concentrations in the lungs. These data imply that an IL-17 signaling pattern contributes to initiation as well as to control the inflammatory response in *Coccidioides* infection since this cytokine controls neutrophil expansion via G-CSF and CXCL1 and induces CCL2/MCP-1 production as well as CCL3/MIP-1 α . Vaccination also showed a population of CD8⁺ IFN- γ ⁺ T cells in the lungs of immunized animals and that these cells can act synergistically with CD4⁺ T cells [106]. The importance of Th17 signaling was observed in the lung homogenate of mice

vaccinated with ΔT . In fact, the period post-challenge showed a predominance of Th2 and Th17 cytokines, followed by a decrease in Th2 levels and a progressive increase in Th1 levels, with the end of Th17 predominance. The use of knock-out mice helped to elucidate this issue: vaccinated IL-17^{-/-} mice were partially protected against lethal lung infection by *Coccidioides*, while IL-4^{-/-} and IFN- γ ^{-/-} mice were not protected, confirming that the vaccine should induce Th17 signaling pattern to be effective [106].

In search for a durable immune response, the ΔT vaccine was conjugated to the EP67 adjuvant, a biologically active agonist of the C-terminal region of the human complement, C5a. The decapeptide adjuvant induces release of Th1 cytokines from APCs and enhances processing and antigen presentation by DCs in humans [72]. The vaccine-EP67 conjugation showed small inflammatory infiltrate in the lung when compared with vaccine alone. Conjugation increased the expression of MHC class II molecules on the cell surface, especially of DCs, when compared with animals vaccinated only with ΔT , together with increased expression of costimulatory molecules (CD80/86 and CD40). In vitro tests with splenocytes from mice vaccinated with the conjugate and recall with *Coccidioides* showed a significant increase of IL-2 and IFN- γ in the supernatant, when compared with splenocytes from animals vaccinated only with the ΔT vaccine. There was also high T-bet expression in the mice vaccinated with the conjugate, showing promotion of differentiation of the Th1 response and dominant CD4⁺ IL-17⁺ [110].

Reactive T cell recombinant antigens eliciting protective and durable immune response against lethal *Coccidioides* infection have been studied for a vaccine. The multicomponent extract of the *C. posadasii* cell wall was used in an attempt to identify these antigens. The *C. posadasii* spherules wall isolate was used as a vaccine, conjugated with the ODN CpG adjuvant. A robust protective response in C57Bl/6 mice was elicited against *C. posadasii* lethal lung challenge resulting in 90% of survivors 50 days after the challenge. An amino acid sequence of a polypeptide revealed similarity with aspartyl protease from other filamentous fungi and was termed Pep1 [73]. ELISPOT analysis of CD90⁺ T cells from the splenocytes of C57Bl/6 mice and HLA-DR4 transgenic mice vaccinated with recombinant rPep1 and recall with rPep1, produced high levels of IFN- γ , a further evidence that the antigen stimulates a potent immune response for Th1 signaling. Most of the surviving mice had their lungs pathogen-cleared 90 days after challenge, indicating that the cellular immune response is durable [111].

Other proteins from the parasitic cell seemed also to have been revealed among them the putative proteins phospholipase B (Plb) and alpha-mannosidase (Amn1). Vaccination of the C57Bl/6 mice with the multivalent recombinant Pep1, Plb, and Amn1 conjugated to ODN CpG resulted in enhanced survival of the mice infected with *C. posadasii* and showed

improved clearance of the pathogen in this murine coccidioidomycosis model [112].

Using a computational analysis of the primary structure of each of the three T cell reactive proteins, a recombinant epitope-based vaccine (rEBV) was created. The vaccine consisted of Pep-1-P1, Pep2-P2, Amn1-P10, Amn1-P11, and Plb-P6, five epitopes based on their affinity for HLA-DR4 molecules. Assays using splenocytes from mice vaccinated with rEBV and ODN CpG and infected, when a recall with rEBV was made, produced high levels of IL-2 and IFN- γ , Th1 cytokines, and high expression of IL-17A indicating the activation of Th17 signaling. The protective immune response was observed by the clonal expansion of Th1 (CD4⁺IFN- γ ⁺) and Th17A (CD4⁺IL-17A⁺) cells in the lungs of mice, 9 days after the challenge and reduction of the CFU of mice vaccinated with rEBV and CpG ODN [74].

Glucan particles (GP) were used as an adjuvant to rEBV vaccine in the search for a protective and long-lasting immune response against coccidioidomycosis. Glucan particles, prepared from yeast cell wall (*Saccharomyces cerevisiae*) rich in β -1,3-glucan were effective as an adjuvant and efficient delivery agent [113]. These particles are hollow, small, can be filled with protein antigen and can be easily captured. Because they are rich in β -glucans they are recognized by Dectin-1, a receptor expressed on the surface of host phagocytes as DCs, macrophages, and neutrophils and induce cytokines involved in Th17 signaling pattern [113–115]. High levels of IL-17A and IFN- γ were observed in splenocyte cultures of mice vaccinated with rEBV plus GP plus OVA, increased number of CD4⁺ IFN- γ ⁺ and CD4⁺ IL-17A⁺ T cells in the lungs of mice vaccinated with rEBV plus GP plus OVA and infected as compared with mice vaccinated with rEBV and ODN CpG. Better results were also obtained for fungal clearance, histopathology and animal survival [74].

All of the information discussed above is summarized in Table 1.

Final Remarks

The development of prophylactic and/or therapeutic vaccines faces many difficulties. In addition to several limitations of antifungal treatment, the major challenge is to find a vaccine that can induce an effective immune response in immunosuppressed patients. The emergence of strains resistant to antifungal drugs and the increase of immunosuppressed populations aggravate this situation. Over the last few years, many researchers have struggled to find vaccines that may reduce the morbidity and mortality of patients with fungal infections, but so far, few prototypes of successful treatment have emerged from clinical trials. Accumulated knowledge, however, on the pathogen-host relationship and the understanding

of the immune response to these microorganisms leave us closer to this goal.

One of the major challenges for the development of vaccines against fungal infections is that these microorganisms mostly affect immunocompromised individuals. Generally, CD4⁺ T cells are essential to mediate protection against systemic mycoses, but patients deficient in these cells do not respond efficiently to antifungal treatment. Alternatively, CD8⁺ T cells are a promising strategy, since the protective immunity mediated by these cells has already been documented against different fungal infections, among them PCM, histoplasmosis and blastomycosis.

Another experimentally tested procedure is the use of vaccines in conjunction with antifungal therapy aiming at decreasing treatment toxicity and therapy period. As discussed previously, the prolonged time of antifungal therapy against systemic mycoses may lead to serious relapses, with great impact on health care. Studies focusing on the use of therapeutic vaccines associated with antifungal agents are, therefore, to be highlighted.

Finally, since many fungal infections are still largely neglected, development of therapeutic vaccines has to overcome scientific and health-policy obstacles to assure convenient financial investment allowing for progress in this area.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

- Iannitti RG, Carvalho A, Romani L. From memory to antifungal vaccine design. *Trends Immunol.* 2012;33:467–74.
- Parente-Rocha JA, Bailão AM, Amaral AC, Taborda CP, Pancez JD, Borges CL, et al. Antifungal resistance, metabolic routes as drug targets, and new antifungal agents: an overview about endemic dimorphic fungi. *Mediat Inflamm.* 2017;2017:9870679.
- Medici NP, Del Poeta M. New insights on the development of fungal vaccines: from immunity to recent challenges. *Mem Inst Oswaldo Cruz.* 2015;110:966–73.
- Goughenour KD, Rappleye CA. Antifungal therapeutics for dimorphic fungal pathogens. *Virulence.* 2017;8:211–21.
- Turissini DA, Gomez OM, Teixeira MM, McEwen JG, Matute DR. Species boundaries in the human pathogen *Paracoccidioides*. *Fungal Genet Biol.* 2017;106:9–25.
- Martinez R. Epidemiology of *Paracoccidioidomycosis*. *Rev Inst Med Trop Sao Paulo.* 2015;57(Suppl 19):11–20.
- Bocca AL, Amaral AC, Teixeira MM, Sato PK, Sato P, Shikanai-Yasuda MA, et al. *Paracoccidioidomycosis*: eco-epidemiology, taxonomy and clinical and therapeutic issues. *Future Microbiol.* 2013;8:1177–91.
- Colombo AL, Tobón A, Restrepo A, Queiroz-Telles F, Nucci M. Epidemiology of endemic systemic fungal infections in Latin America. *Med Mycol.* 2011;49:785–98.
- Pan B, Chen M, Pan W, Liao W. Histoplasmosis: a new endemic fungal infection in China? Review and analysis of cases. *Mycoses.* 2013;56:212–21.
- Antinori S. *Histoplasma capsulatum*: more widespread than previously thought. *Am J Trop Med Hyg.* 2014;90:982–3.
- Vallabhaneni S, Mody RK, Walker T, Chiller T. The global burden of fungal diseases. *Infect Dis Clin N Am.* 2016;30:1–11.
- Horwath MC, Fecher RA, Deepe GS. *Histoplasma capsulatum*, lung infection and immunity. *Future Microbiol.* 2015;10:967–75.
- Stockamp NW, Thompson GR. *Coccidioidomycosis*. *Infect Dis Clin N Am.* 2016;30:229–46.
- Brown J, Benedict K, Park BJ, Thompson GR. *Coccidioidomycosis*: epidemiology. *Clin Epidemiol.* 2013;5:185–97.
- McBride JA, Gauthier GM, Klein BS. Clinical manifestations and treatment of blastomycosis. *Clin Chest Med.* 2017;38:435–49.
- Beardsley J, Halliday CL, Chen SC, Sorrell TC. Responding to the emergence of antifungal drug resistance: perspectives from the bench and the bedside. *Future Microbiol.* 2018;13:1175–91.
- White TC, Marr KA, Bowden RA. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. *Clin Microbiol Rev.* 1998;11:382–402.
- de Oliveira HC, da Silva JF, Scorzoni L, Marcos CM, Rossi SA, de Paula E Silva AC, et al. Importance of adhesins in virulence of *Paracoccidioides* spp. *Front Microbiol.* 2015;6:303.
- Thompson GR, Rendon A, Ribeiro Dos Santos R, Queiroz-Telles F, Ostrosky-Zeichner L, Azie N, et al. Isavuconazole treatment of cryptococcosis and dimorphic mycoses. *Clin Infect Dis.* 2016;63:356–62.
- Travassos LR, Taborda CP. Linear epitopes of *Paracoccidioides brasiliensis* and other fungal agents of human systemic mycoses as vaccine candidates. *Front Immunol.* 2017;8:224.
- Scorzoni L, de Paula E Silva AC, Marcos CM, Assato PA, de Melo WC, de Oliveira HC, et al. Antifungal therapy: new advances in the understanding and treatment of mycosis. *Front Microbiol.* 2017;8:36.
- Demicheli MC, Reis BS, Goes AM, de Andrade AS. *Paracoccidioides brasiliensis*: attenuation of yeast cells by gamma irradiation. *Mycoses.* 2006;49:184–9.
- Demicheli MC, Goes AM, de Andrade AS. Ultrastructural changes in *Paracoccidioides brasiliensis* yeast cells attenuated by gamma irradiation. *Mycoses.* 2007;50:397–402.
- Puccia R, Schenkman S, Gorin PA, Travassos LR. Exocellular components of *Paracoccidioides brasiliensis*: identification of a specific antigen. *Infect Immun.* 1986;53:199–206.
- Puccia R, Travassos LR. 43-kilodalton glycoprotein from *Paracoccidioides brasiliensis*: immunochemical reactions with sera from patients with *paracoccidioidomycosis*, histoplasmosis, or Jorge Lobo's disease. *J Clin Microbiol.* 1991;29:1610–5.
- Rodrigues EG, Travassos LR. Nature of the reactive epitopes in *Paracoccidioides brasiliensis* polysaccharide antigen. *J Med Vet Mycol.* 1994;32:77–81.

27. Saraiva EC, Altemani A, Franco MF, Unterkircher CS, Camargo ZP. Paracoccidioides brasiliensis-gp43 used as paracoccidioidin. *J Med Vet Mycol.* 1996;34:155–61.
28. Taborda CP, Juliano MA, Puccia R, Franco M, Travassos LR. Mapping of the T-cell epitope in the major 43-kilodalton glycoprotein of *Paracoccidioides brasiliensis* which induces a Th-1 response protective against fungal infection in BALB/c mice. *Infect Immun.* 1998;66:786–93.
29. Iwai LK, Yoshida M, Sidney J, Shikanai-Yasuda MA, Goldberg AC, Juliano MA, et al. In silico prediction of peptides binding to multiple HLA-DR molecules accurately identifies immunodominant epitopes from gp43 of *Paracoccidioides brasiliensis* frequently recognized in primary peripheral blood mononuclear cell responses from sensitized individuals. *Mol Med.* 2003;9:209–19.
30. Iwai LK, Yoshida M, Sadahiro A, da Silva WR, Marin ML, Goldberg AC, et al. T-cell recognition of *Paracoccidioides brasiliensis* gp43-derived peptides in patients with paracoccidioidomycosis and healthy individuals. *Clin Vaccine Immunol.* 2007;14:474–6.
31. Travassos LR, Rodrigues EG, Iwai LK, Taborda CP. Attempts at a peptide vaccine against paracoccidioidomycosis, adjuvant to chemotherapy. *Mycopathologia.* 2008;165:341–52.
32. Travassos LR, Taborda CP. New advances in the development of a vaccine against paracoccidioidomycosis. *Front Microbiol.* 2012;3:212.
33. Taborda CP, Urán ME, Nosanchuk JD, Travassos LR. Paracoccidioidomycosis: challenges in the development of a vaccine against an endemic mycosis in the Americas. *Rev Inst Med Trop Sao Paulo.* 2015;57(Suppl 19):21–4.
34. Marques AF, da Silva MB, Juliano MA, Travassos LR, Taborda CP. Peptide immunization as an adjuvant to chemotherapy in mice challenged intratracheally with virulent yeast cells of *Paracoccidioides brasiliensis*. *Antimicrob Agents Chemother.* 2006;50:2814–9.
35. Marques AF, da Silva MB, Juliano MA, Munhöz JE, Travassos LR, Taborda CP. Additive effect of P10 immunization and chemotherapy in anergic mice challenged intratracheally with virulent yeasts of *Paracoccidioides brasiliensis*. *Microbes Infect.* 2008;10:1251–8.
36. Travassos LR, Taborda CP. Paracoccidioidomycosis vaccine. *Hum Vaccin Immunother.* 2012;8:1450–3.
37. Amaral AC, Marques AF, Muñoz JE, Bocca AL, Simioni AR, Tedesco AC, et al. Poly(lactic acid-glycolic acid) nanoparticles markedly improve immunological protection provided by peptide P10 against murine paracoccidioidomycosis. *Br J Pharmacol.* 2010;159:1126–32.
38. Muñoz JE, Luft VD, Amorim J, Magalhães A, Thomaz L, Nosanchuk JD, et al. Immunization with P10 peptide increases specific immunity and protects immunosuppressed BALB/c mice infected with virulent yeasts of *Paracoccidioides brasiliensis*. *Mycopathologia.* 2014;178:177–88.
39. de Amorim J, Magalhães A, Muñoz JE, Rittner GM, Nosanchuk JD, Travassos LR, et al. DNA vaccine encoding peptide P10 against experimental paracoccidioidomycosis induces long-term protection in presence of regulatory T cells. *Microbes Infect.* 2013;15:181–91.
40. Rittner GM, Muñoz JE, Marques AF, Nosanchuk JD, Taborda CP, Travassos LR. Therapeutic DNA vaccine encoding peptide P10 against experimental paracoccidioidomycosis. *PLoS Negl Trop Dis.* 2012;6:e1519.
41. Ribeiro AM, Bocca AL, Amaral AC, Souza AC, Faccioli LH, Coelho-Castelo AA, et al. HSP65 DNA as therapeutic strategy to treat experimental paracoccidioidomycosis. *Vaccine.* 2010;28:1528–34.
42. Ribeiro AM, Souza AC, Amaral AC, Vasconcelos NM, Jeronimo MS, Carneiro FP, et al. Nanobiotechnological approaches to delivery of DNA vaccine against fungal infection. *J Biomed Nanotechnol.* 2013;9:221–30.
43. Magalhães A, Ferreira KS, Almeida SR, Nosanchuk JD, Travassos LR, Taborda CP. Prophylactic and therapeutic vaccination using dendritic cells primed with peptide 10 derived from the 43-kilodalton glycoprotein of *Paracoccidioides brasiliensis*. *Clin Vaccine Immunol.* 2012;19:23–9.
44. Silvana dos Santos S, Ferreira KS, Almeida SR. Paracoccidioides brasiliensis-induced migration of dendritic cells and subsequent T-cell activation in the lung-draining lymph nodes. *PLoS One.* 2011;6:e19690.
45. Silva LBR, Dias LS, Rittner GM, Muñoz JE, Souza ACO, Nosanchuk JD, et al. Dendritic cells primed with *Paracoccidioides brasiliensis* peptide P10 are therapeutic in immunosuppressed mice with paracoccidioidomycosis. *Front Microbiol.* 2017;8:1057.
46. Ferreira KS, Lopes JD, Almeida SR. Regulation of T helper cell differentiation in vivo by GP43 from *Paracoccidioides brasiliensis* provided by different antigen-presenting cells. *Scand J Immunol.* 2003;58:290–7.
47. Thind SK, Taborda CP, Nosanchuk JD. Dendritic cell interactions with *Histoplasma* and *Paracoccidioides*. *Virulence.* 2015;6:424–32.
48. Fernandes VC, Martins EM, Boeloni JN, Coitinho JB, Serakides R, Goes AM. The combined use of *Paracoccidioides brasiliensis* Pb40 and Pb27 recombinant proteins enhances chemotherapy effects in experimental paracoccidioidomycosis. *Microbes Infect.* 2011;13:1062–72.
49. Souto JT, Figueiredo F, Furlanetto A, Pfeffer K, Rossi MA, Silva JS. Interferon-gamma and tumor necrosis factor-alpha determine resistance to *Paracoccidioides brasiliensis* infection in mice. *Am J Pathol.* 2000;156:1811–20.
50. Adams DO. The granulomatous inflammatory response. A review. *Am J Pathol.* 1976;84:164–92.
51. Tracey KJ, Cerami A. Tumor necrosis factor, other cytokines and disease. *Annu Rev Cell Biol.* 1993;9:317–43.
52. Holanda RA, Muñoz JE, Dias LS, Silva LBR, Santos JRA, Pagliari S, et al. Recombinant vaccines of a CD4+ T-cell epitope promote efficient control of *Paracoccidioides brasiliensis* burden by restraining primary organ infection. *PLoS Negl Trop Dis.* 2017;11:e0005927.
53. Jannuzzi GP, Souza NA, Françoiso KS, Pereira RH, Santos RP, Kaihami GH, et al. Therapeutic treatment with scFv-PLGA nanoparticles decreases pulmonary fungal load in a murine model of paracoccidioidomycosis. *Microbes Infect.* 2018;20:48–56.
54. de Mattos Grosso D, de Almeida SR, Mariano M, Lopes JD. Characterization of gp70 and anti-gp70 monoclonal antibodies in *Paracoccidioides brasiliensis* pathogenesis. *Infect Immun.* 2003;71:6534–42.
55. Xander P, Vigna AF, Feitosa LS, Pugliese L, Bailão AM, Soares CM, et al. A surface 75-kDa protein with acid phosphatase activity recognized by monoclonal antibodies that inhibit *Paracoccidioides brasiliensis* growth. *Microbes Infect.* 2007;9:1484–92.
56. Buissa-Filho R, Puccia R, Marques AF, Pinto FA, Muñoz JE, Nosanchuk JD, et al. The monoclonal antibody against the major diagnostic antigen of *Paracoccidioides brasiliensis* mediates immune protection in infected BALB/c mice challenged intratracheally with the fungus. *Infect Immun.* 2008;76:3321–8.
57. Straus AH, Freymüller E, Travassos LR, Takahashi HK. Immunochemical and subcellular localization of the 43 kDa glycoprotein antigen of *Paracoccidioides brasiliensis* with monoclonal antibodies. *J Med Vet Mycol.* 1996;34:181–6.
58. Thomaz L, Nosanchuk JD, Rossi DC, Travassos LR, Taborda CP. Monoclonal antibodies to heat shock protein 60 induce a

- protective immune response against experimental *Paracoccidioides lutzii*. *Microbes Infect.* 2014;16:788–95.
59. Gómez AM, Rhodes JC, Deepe GS. Antigenicity and immunogenicity of an extract from the cell wall and cell membrane of *Histoplasma capsulatum* yeast cells. *Infect Immun.* 1991;59:330–6.
 60. Gomez FJ, Gomez AM, Deepe GS. Protective efficacy of a 62-kilodalton antigen, HIS-62, from the cell wall and cell membrane of *Histoplasma capsulatum* yeast cells. *Infect Immun.* 1991;59:4459–64.
 61. Gomez FJ, Allendoerfer R, Deepe GS. Vaccination with recombinant heat shock protein 60 from *Histoplasma capsulatum* protects mice against pulmonary histoplasmosis. *Infect Immun.* 1995;63:2587–95.
 62. Scheckelhoff M, Deepe GS. The protective immune response to heat shock protein 60 of *Histoplasma capsulatum* is mediated by a subset of V beta 8.1/8.2+ T cells. *J Immunol.* 2002;169:5818–26.
 63. Deepe GS, Gibbons R. Protective efficacy of H antigen from *Histoplasma capsulatum* in a murine model of pulmonary histoplasmosis. *Infect Immun.* 2001;69:3128–34.
 64. Sá-Nunes A, Medeiros AI, Nicolette R, Frantz FG, Panunto-Castelo A, Silva CL, et al. Efficacy of cell-free antigens in evaluating cell immunity and inducing protection in a murine model of histoplasmosis. *Microbes Infect.* 2005;7:584–92.
 65. Deepe GS, Buesing WR, Ostroff GR, Abraham A, Specht CA, Huang H, et al. Vaccination with an alkaline extract of *Histoplasma capsulatum* packaged in glucan particles confers protective immunity in mice. *Vaccine.* 2018;36:3359–67.
 66. Klein BS, Jones JM. Isolation, purification, and radiolabeling of a novel 120-kD surface protein on *Blastomyces dermatitidis* yeasts to detect antibody in infected patients. *J Clin Invest.* 1990;85:152–61.
 67. Klein BS, Sondel PM, Jones JM. WI-1, a novel 120-kilodalton surface protein on *Blastomyces dermatitidis* yeast cells, is a target antigen of cell-mediated immunity in human blastomycosis. *Infect Immun.* 1992;60:4291–300.
 68. Wüthrich M, Gern B, Hung CY, Ersland K, Rocco N, Pick-Jacobs J, et al. Vaccine-induced protection against 3 systemic mycoses endemic to North America requires Th17 cells in mice. *J Clin Invest.* 2011;121:554–68.
 69. Wang H, LeBert V, Hung CY, Galles K, Saijo S, Lin X, et al. C-type lectin receptors differentially induce th17 cells and vaccine immunity to the endemic mycosis of North America. *J Immunol.* 2014;192:1107–19.
 70. Abuodeh RO, Shubitz LF, Siegel E, Snyder S, Peng T, Orsbom KI, et al. Resistance to *Coccidioides immitis* in mice after immunization with recombinant protein or a DNA vaccine of a proline-rich antigen. *Infect Immun.* 1999;67:2935–40.
 71. Xue J, Chen X, Selby D, Hung CY, Yu JJ, Cole GT. A genetically engineered live attenuated vaccine of *Coccidioides posadasii* protects BALB/c mice against coccidioidomycosis. *Infect Immun.* 2009;77:3196–208.
 72. Morgan EL, Thoman ML, Sanderson SD, Phillips JA. A novel adjuvant for vaccine development in the aged. *Vaccine.* 2010;28:8275–9.
 73. Johnson SM, Kerekes KM, Zimmermann CR, Williams RH, Pappagianis D. Identification and cloning of an aspartyl proteinase from *Coccidioides immitis*. *Gene.* 2000;241:213–22.
 74. Hurtgen BJ, Hung CY, Ostroff GR, Levitz SM, Cole GT. Construction and evaluation of a novel recombinant T cell epitope-based vaccine against coccidioidomycosis. *Infect Immun.* 2012;80:3960–74.
 75. Nosanchuk JD, Steenbergen JN, Shi L, Deepe GS, Casadevall A. Antibodies to a cell surface histone-like protein protect against *Histoplasma capsulatum*. *J Clin Invest.* 2003;112:1164–75.
 76. Guimarães AJ, Frases S, Gomez FJ, Zancopé-Oliveira RM, Nosanchuk JD. Monoclonal antibodies to heat shock protein 60 alter the pathogenesis of *Histoplasma capsulatum*. *Infect Immun.* 2009;77:1357–67.
 77. Wüthrich M, Klein BS. Investigation of anti-WI-1 adhesin antibody-mediated protection in experimental pulmonary blastomycosis. *J Infect Dis.* 2000;181:1720–8.
 78. Deepe GS, Durose GG. Immunobiological activity of recombinant H antigen from *Histoplasma capsulatum*. *Infect Immun.* 1995;63:3151–7.
 79. Deepe GS, Gibbons RS. Cellular and molecular regulation of vaccination with heat shock protein 60 from *Histoplasma capsulatum*. *Infect Immun.* 2002;70:3759–67.
 80. Allen HL, Deepe GS. B cells and CD4-CD8- T cells are key regulators of the severity of reactivation histoplasmosis. *J Immunol.* 2006;177:1763–71.
 81. Chapman SW, Dismukes WE, Proia LA, Bradsher RW, Pappas PG, Threlkeld MG, et al. Clinical practice guidelines for the management of blastomycosis: 2008 update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2008;46:1801–12.
 82. Wüthrich M, Deepe GS, Klein B. Adaptive immunity to fungi. *Annu Rev Immunol.* 2012;30:115–48.
 83. Romani L. Immunity to fungal infections. *Nat Rev Immunol.* 2011;11:275–88.
 84. Chang WL, Audet RG, Aizenstein BD, Hogan LH, DeMars RI, Klein BS. T-cell epitopes and human leukocyte antigen restriction elements of an immunodominant antigen of *Blastomyces dermatitidis*. *Infect Immun.* 2000;68:502–10.
 85. Wüthrich M, Filutowicz HI, Warner T, Klein BS. Requisite elements in vaccine immunity to *Blastomyces dermatitidis*: plasticity uncovers vaccine potential in immune-deficient hosts. *J Immunol.* 2002;169:6969–76.
 86. Wüthrich M, Filutowicz HI, Warner T, Deepe GS, Klein BS. Vaccine immunity to pathogenic fungi overcomes the requirement for CD4 help in exogenous antigen presentation to CD8+ T cells: implications for vaccine development in immune-deficient hosts. *J Exp Med.* 2003;197:1405–16.
 87. Nanjappa SG, Heninger E, Wüthrich M, Sullivan T, Klein B. Protective antifungal memory CD8(+) T cells are maintained in the absence of CD4(+) T cell help and cognate antigen in mice. *J Clin Invest.* 2012;122:987–99.
 88. Zelante T, De Luca A, D'Angelo C, Moretti S, Romani L. IL-17/Th17 in anti-fungal immunity: what's new? *Eur J Immunol.* 2009;39:645–8.
 89. Deepe GS, Gibbons RS. Interleukins 17 and 23 influence the host response to *Histoplasma capsulatum*. *J Infect Dis.* 2009;200:142–51.
 90. Rogers NC, Slack EC, Edwards AD, Nolte MA, Schulz O, Schweighoffer E, et al. Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity.* 2005;22:507–17.
 91. Underhill DM, Rossmagle E, Lowell CA, Simmons RM. Dectin-1 activates Syk tyrosine kinase in a dynamic subset of macrophages for reactive oxygen production. *Blood.* 2005;106:2543–50.
 92. Gross O, Gewies A, Finger K, Schäfer M, Sparwasser T, Peschel C, et al. Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature.* 2006;442:651–6.
 93. Viriyakosol S, Jimenez MP, Gurney MA, Ashbaugh ME, Fierer J. Dectin-1 is required for resistance to coccidioidomycosis in mice. *MBio.* 2013;4:e00597–12.
 94. Wüthrich M, Hung CY, Gern BH, Pick-Jacobs JC, Galles KJ, Filutowicz HI, et al. A TCR transgenic mouse reactive with multiple systemic dimorphic fungi. *J Immunol.* 2011;187:1421–31.
 95. Ben-Sasson SZ, Hu-Li J, Quiel J, Cauchetaux S, Ratner M, Shapira I, et al. IL-1 acts directly on CD4 T cells to enhance their

- antigen-driven expansion and differentiation. *Proc Natl Acad Sci U S A*. 2009;106:7119–24.
96. Ben-Sasson SZ, Hogg A, Hu-Li J, Wingfield P, Chen X, Crank M, et al. IL-1 enhances expansion, effector function, tissue localization, and memory response of antigen-specific CD8 T cells. *J Exp Med*. 2013;210:491–502.
 97. Wüthrich M, LeBert V, Galles K, Hu-Li J, Ben-Sasson SZ, Paul WE, et al. Interleukin 1 enhances vaccine-induced antifungal T-helper 17 cells and resistance against *Blastomyces dermatitidis* infection. *J Infect Dis*. 2013;208:1175–82.
 98. Newman SL, Chaturvedi S, Klein BS. The WI-1 antigen of *Blastomyces dermatitidis* yeasts mediates binding to human macrophage CD11b/CD18 (CR3) and CD14. *J Immunol*. 1995;154:753–61.
 99. Wüthrich M, Chang WL, Klein BS. Immunogenicity and protective efficacy of the WI-1 adhesin of *Blastomyces dermatitidis*. *Infect Immun*. 1998;66:5443–9.
 100. Wüthrich M, Filutowicz HI, Klein BS. Mutation of the WI-1 gene yields an attenuated *blastomyces dermatitidis* strain that induces host resistance. *J Clin Invest*. 2000;106:1381–9.
 101. Engelthaler DM, Roe CC, Hepp CM, Teixeira M, Driebe EM, Schupp JM, et al. Local population structure and patterns of Western hemisphere dispersal for *Coccidioides* spp., the fungal cause of valley fever. *MBio*. 2016;7:e00550–16.
 102. Sharpton TJ, Stajich JE, Rounsley SD, Gardner MJ, Wortman JR, Jordan VS, et al. Comparative genomic analyses of the human fungal pathogens *Coccidioides* and their relatives. *Genome Res*. 2009;19:1722–31.
 103. Fisher MC, Koenig GL, White TJ, Taylor JW. Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognized as the non-California population of *Coccidioides immitis*. *Mycologia*. 2002;94:73–84.
 104. Kirkland TN, Fierer J. *Coccidioidomycosis*: a reemerging infectious disease. *Emerg Infect Dis*. 1996;2:192–9.
 105. Kirkland TN. The quest for a vaccine against *Coccidioidomycosis*: a neglected disease of the Americas. *J Fungi (Basel)* 2016;2.
 106. Hung CY, Gonzalez A, Wüthrich M, Klein BS, Cole GT. Vaccine immunity to *coccidioidomycosis* occurs by early activation of three signal pathways of T helper cell response (Th1, Th2, and Th17). *Infect Immun*. 2011;79:4511–22.
 107. Fierer J, Waters C, Walls L. Both CD4+ and CD8+ T cells can mediate vaccine-induced protection against *Coccidioides immitis* infection in mice. *J Infect Dis*. 2006;193:1323–31.
 108. Magee DM, Friedberg RL, Woitaske MD, Johnston SA, Cox RA. Role of B cells in vaccine-induced immunity against *coccidioidomycosis*. *Infect Immun*. 2005;73:7011–3.
 109. Peng T, Shubitz L, Simons J, Perrill R, Orsborn KI, Galgiani JN. Localization within a proline-rich antigen (Ag2/PRA) of protective antigenicity against infection with *Coccidioides immitis* in mice. *Infect Immun*. 2002;70:3330–5.
 110. Hung CY, Hurtgen BJ, Bellecourt M, Sanderson SD, Morgan EL, Cole GT. An agonist of human complement fragment C5a enhances vaccine immunity against *Coccidioides* infection. *Vaccine*. 2012;30:4681–90.
 111. Tarcha EJ, Basrur V, Hung CY, Gardner MJ, Cole GT. A recombinant aspartyl protease of *Coccidioides posadasii* induces protection against pulmonary *coccidioidomycosis* in mice. *Infect Immun*. 2006;74:516–27.
 112. Tarcha EJ, Basrur V, Hung CY, Gardner MJ, Cole GT. Multivalent recombinant protein vaccine against *coccidioidomycosis*. *Infect Immun*. 2006;74:5802–13.
 113. Huang H, Ostroff GR, Lee CK, Specht CA, Levitz SM. Robust stimulation of humoral and cellular immune responses following vaccination with antigen-loaded beta-glucan particles. *MBio*. 2010;1.
 114. Huang H, Ostroff GR, Lee CK, Agarwal S, Ram S, Rice PA, et al. Relative contributions of dectin-1 and complement to immune responses to particulate β -glucans. *J Immunol*. 2012;189:312–7.
 115. Huang H, Ostroff GR, Lee CK, Specht CA, Levitz SM. Characterization and optimization of the glucan particle-based vaccine platform. *Clin Vaccine Immunol*. 2013;20:1585–91.

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