Vaccine immunity against fungal infections
Som G. Nanjappa¹ and Bruce S. Klein¹,²,³

Addresses
¹ Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI 53792, United States
² Department of Internal Medicine, University of Wisconsin School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI 53792, United States
³ Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI 53792, United States

Corresponding authors: Nanjappa, Som G. (snanjappa@pediatrics.wisc.edu) and Klein, Bruce S. (bsklein@wisc.edu)

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CD4⁺ T cells play a major role in mediating resistance to fungal infection, hence the higher incidences in individuals with reduced CD4⁺ T cell numbers or function. CD4⁺ T cells confer resistance through secretion of T-helper (Th) 1 or Th17 cytokines such as IFNγ, TNFα, GM-CSF and IL-17A, respectively, which activate neutrophils, macrophages, dendritic cells and inflammatory monocytes for fungal killing and clearance; and by activating B cells to secrete protective antibodies [1,2]. IL-17 is integral for protection against a number of fungal pathogens, although its role is debated in animal models of aspergillosis and gastric candidiasis, where it can exacerbate disease [1,2,4,5,6,7,8]. Fungal vaccine candidates have focused mainly on stimulating CD4⁺ T cells and B cells and protection mediated by these defenses. A growing appreciation for the role of CD8⁺ T cells in anti-fungal immunity has offered an avenue to exploit alternative vaccine strategies designed for individuals with CD4⁺ T cell lymphopenia syndromes like in AIDS. In this review, we address recent developments in fungal vaccines and discuss new insights about immune mechanisms that govern protection against fungi, including the underappreciated role of CD8⁺ T cell immunity against fungal infections.

Vaccines against the fungi
In recent years, a number of fungal vaccine candidates have been reported, refined and tested in animal models for their safety, immunogenicity and efficacy and at least two of them are now under study in human clinical trials. Many are listed in Table 1; several are emphasized below.

Vaccination against Candida; Candida albicans is most commonly found as a commensal on mucosal surfaces in the gut and vagina. However, C. albicans can cause mucocutaneous infections of the oropharynx, skin, nails and vagina; and produce life-threatening bloodstream infections in immune suppressed hosts or when anatomical barriers are breached. The major risk factors for invasive candidiasis are medical interventions such as those following transplantation of solid organs, hematopoietic stem cells or bone marrow; surgeries; and use of IV catheters. C. albicans is most common in these settings.
although non-albicans Candida sp. such as C. glabrata have recently emerged [9].

Vaccine immunity against Candida infection in animal models is mediated by Th1 and Th17 cells, and by antibodies (Table 1). An investigational candidate vaccine containing rAsp5p-N (NDV-3), which is directed against Candida and also Staphylococcus aureus, has been tested for safety and immunogenicity in volunteers in a Phase I clinical trial, and efficacy studies are now being planned. Another candidate vaccine containing rSap2p was found to be well tolerated and effective at low doses in inducing specific antibodies and B cell memory in women with recurrent vulvovaginitis in a European clinical trial [10].

**Vaccination against pulmonary mycoses.** Pulmonary mycoses are acquired by inhalation of spores or mycelial fragments. Clinical manifestations vary from mild, self-limiting lung infections to fulminant ARDS, or life-threatening, disseminated infections during immunosuppression.

Aspergillus sp. can cause acute and chronic pneumonia, allergic bronchopulmonary aspergillosis, aspergilloma and invasive aspergillosis. Experimental vaccines have been shown to mediate protection by inducing strong Th1 responses (Table 1). Th2 responses have been associated with allergic reactions and defective fungal clearance, while Th17 responses during primary infection can be pathological. Immunity against Aspergillus and other fungi has been observed after vaccination with heat-killed Saccharomyces. Similarly, Crf1 protein induced Aspergillus specific CD4+ T-cell responses in healthy individuals [11], and vaccination with the same Crf1p mediated cross-protection against candidiasis and aspergillosis [12].

Cryptococcus neoformans can cause life-threatening pulmonary and CNS infections in patients with severely compromised immunity such as in AIDS, whereas Cryptococcus gattii infection has recently emerged as a pathogen in previously healthy individuals. Cryptococcus is a leading cause of meningitis in some parts of the world (see below). Candidate vaccines comprising Glucoronoxylomannan (GXM), peptide mimotopes of GXM, cell filtrate antigens (Cnf1) and protein-conjugated laminaran were shown to mediate protection by inducing antibodies and promoting Th1 cell responses (Table 1). Mannosylation of protein antigen has been shown to greatly promote anti-cryptococcal Th1 and CD8+ T cell responses and protective immunity [13].

Pneumocystis pneumonia is generally restricted to immunocompromised patients and carries a high mortality rate. Antibody and CD8+ T cells have been shown to play a role in vaccine-induced resistance to Pneumocystis mediated by Kexin vaccination in immune deficient mice [14]. DNA vaccines have shown some promise in murine models, with protection likely mediated by antibodies and Th1 cell responses [15,16].

Thermally dimorphic fungi grow as sporulating molds in soil. After spores or mycelial fragments are inhaled, they convert to parasitic yeast or spherules that initiate infection and can disseminate. An attenuated live vaccine lacking the virulence factor Bad-1 was developed against blastomycosis; vaccine immunity is primarily mediated by Th1 and Th17 cells, but not by antibodies [2]. Protective CD4+ T cells raised with that vaccine have been found to mediate broad protection against blastomycosis, as well as histoplasmosis and coccidioidomycosis, suggesting a conserved protective antigen and the prospect of a 'pan-fungal' vaccine [6]. Th17 cells were necessary and sufficient in mediating vaccine immunity to these mycoses, supporting growing evidence that IL-17A confers resistance to fungal infections.

Vaccination with live Histoplasma confers protection against lethal pulmonary infection in a murine model [6]. Vaccination with rHSP60 is also capable of eliciting protective immunity via CD4+ T-cells and the action of IFN-γ [17]. A killed spherule vaccine against coccidioidomycosis failed to provide protection in humans during a Phase 3 clinical trial, but recent work using recombinant Ag2/PRA-CSA as a vaccine in nonhuman primates has shown promise [18]. Likewise, vaccination of mice against Coccidioides using multiple T-cell epitopes induced resistance, which was linked to induction of Th1 and Th17 cytokines [19]. Several protein-based vaccine candidates have been shown to provide therapeutic immunity against paracoccidioidomycosis where Th1 and antibodies played a protective role [20–22].

**CD8+ T-cell immunity against fungal infections.** Most of the existing fungal vaccine candidates have not been studied for immune stimulation of CD8+ T cells in CD4+ T-cell deficient hosts. Fungal infections in CD4+ T cell lymphopenic patients have risen at alarming rates due to the HIV pandemic, and despite antifungal therapy, they can carry a mortality rate of 5-70% depending on the type of fungal infection [23–25]. In a recent survey, cryptococcosis in sub-Saharan Africa produced mortality rates of 50-70%, even surpassing deaths caused by tuberculosis, another major opportunistic infection associated with AIDS [24]. Even though opportunistic fungi frequently emerge in HIV patients, primary systemic fungal pathogens also cause life-threatening disease when effector CD4+ T cells are absent or functionally impaired, signifying the need for alternative strategies of control and prevention in immune compromised patients [26,27]. Hence, understanding the role of CD8+ T cell immunity against fungal infections may aid in the development of fungal vaccines tailored to such patients. Several studies have documented protective immunity mediated by CD8+ T cells against fungal pathogens.
Table 1
A list of fungal vaccine candidates.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Candidate</th>
<th>Subunit/Whole</th>
<th>Immunity</th>
<th>Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus</td>
<td>Asp 16 f</td>
<td>Recombinant/subunit</td>
<td>Th1</td>
<td></td>
<td>[45]</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>Asp 3 f</td>
<td>Recombinant/subunit</td>
<td>Th1</td>
<td></td>
<td>[46]</td>
</tr>
<tr>
<td>Aspergillus, Candida</td>
<td>Pep1p, Gel1p, Crf1, glucans</td>
<td>Recombinant/subunit</td>
<td>Th1</td>
<td></td>
<td>[47]</td>
</tr>
<tr>
<td>Aspergillus, Candida</td>
<td>Cell wall glucanase, Crf1</td>
<td>Recombinant</td>
<td>Th1</td>
<td></td>
<td>[12**]</td>
</tr>
<tr>
<td>Aspergillus, Candida</td>
<td>Heat-killed Saccharomyces cerevisiae (H-KY)</td>
<td>Whole</td>
<td>Heat killed-Whole (Th1, Th2, Th17, Antibodies)?</td>
<td></td>
<td>[48,49]</td>
</tr>
<tr>
<td>Candida Blastomyces, Candida</td>
<td>Attenuated mutant</td>
<td>Recombinant/subunit</td>
<td>Th1, Th17, Antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>Agglutinin-like sequence adhesins (Als1p/Als3p)/Als3p-N (NDV-3)</td>
<td>Whole</td>
<td>Th1, Th17, Antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>Laminaran</td>
<td>Recombinant/subunit</td>
<td>Th1/Th17, Antibodies</td>
<td>Murine</td>
<td>[50**]</td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>Mannann linked to human serum albumin</td>
<td>Subunit</td>
<td>(Candida mannan) based</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>Fba peptide</td>
<td>Recombinant/truncated</td>
<td>Antibodies</td>
<td>Murine</td>
<td></td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>Attenuated mutant</td>
<td>Whole</td>
<td>Th1, Th17, Th2?</td>
<td></td>
<td>[9]</td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>T-cell epitopes</td>
<td>Antigen 2/3/4/proline rich Aga</td>
<td>Antibodies?</td>
<td>Murine</td>
<td>[51]</td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>Glucuronoxylanman (GXM) capsule</td>
<td>Subunit</td>
<td>Antibodies?</td>
<td>Murine</td>
<td>[52]</td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>Peptide Mimotopes of GXM capsule (P13-linked to Tatanus or Diphtheria toxoid)</td>
<td>Subunit/Recombinant</td>
<td>Antibodies</td>
<td>Murine</td>
<td>[53**]</td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>Craf (culture filtrate Ags), Mannoprotein</td>
<td>Subunit/Recombinant</td>
<td>Th1, Antibodies</td>
<td>Murine</td>
<td>[54]</td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>Cell wall membrane</td>
<td>Subunit</td>
<td>Th1, Th17</td>
<td></td>
<td>[55]</td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>Heat-killed rPb27</td>
<td>Recombinant</td>
<td>Antibodies</td>
<td>Murine</td>
<td>[56]</td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>HSP60</td>
<td>Recombinant</td>
<td>Th1</td>
<td></td>
<td>[57]</td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>P10 (peptide)</td>
<td>Subunit</td>
<td>Th1</td>
<td></td>
<td>[58]</td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>55 kDa DNA/p-55</td>
<td>Recombinant/subunit</td>
<td>Antibodies, CD8+ T cells</td>
<td>Murine</td>
<td>[59]</td>
</tr>
<tr>
<td>Candida Cryptococcus</td>
<td>Kexin</td>
<td>Recombinant/subunit</td>
<td>Antibodies, CD8+ T cells</td>
<td>Murine</td>
<td>[60]</td>
</tr>
</tbody>
</table>

* Under study in human clinical trials.

(Table 2), although CD4+ T cells usually exert a more prominent role in immune competent hosts. Even if vaccine-induced immunity is ‘unnatural’, and not otherwise evident in immunologically intact hosts, perhaps the development of fungal vaccines tailored to augment memory CD8+ T cell responses may help combat mycoses-related mortality in at risk individuals. Below, we address this potential alternative avenue in the development of fungal vaccines for CD4+ T-cell lymphopenic patients.

Protective anti-fungal CD8+ T cell responses and resistance can be elicited in the absence of obvious CD4+ T cell help [4*,28–33]. Anti-fungal CD8+ T-cell responses are generally elicited by cross-presentation and MHC class I loading of fungal peptides. Cross-priming of CD8+ T cells can be influenced by host immune status and the nature of the vaccine. In a model of Aspergillus vaccine immunity, cross-priming of CD8+ T cells required endosome alkalinization and autophagy in dendritic cells, which was defective in CGD mice, yet priming of CD4+ T cells by soluble antigen in these mice remained intact, involving distinct and preserved pathways [34**].

Anti-fungal immunity in the absence of CD4+ T cell immunity is mediated by Tc1 (type 1) cells that secrete IFNy, TNFα, and GM-CSF, as well as by the cytotoxic factors perforin, granulysin and granzyme K, suggesting indirect killing (pro-inflammatory cytokine activation of other cells) and direct killing mechanisms. However, like the prominent role played by Th17 cells in intact hosts, recent studies have shown that anti-fungal immunity also can be mediated by Tc17 cells (IL-17A producing CD8+ T cells) in CD4+ T cell deficient hosts [4*,35**]. For example, in a model of blastomycosis, Tc17 cells were...
required for vaccine immunity in the absence of CD4+ T cells [35**]. Similar to other models, IL-12 was involved in Tc1 priming whereas IL-6 and likely IL-1 and IL-23 were required for Tc17 cell responses [35**,36] (Figure 1). Tc17 cell effectors selectively expressed chemokine receptor CCR6, whereas Tc1 cells preferentially expressed CXCR3 and these receptors were required for recruitment into the lungs during pulmonary infection [35**,37**,38]. By contrast to Tc1 cells, Tc17 cells were mainly CD62Llo and CD27int, suggesting an effector memory phenotype [35**]. However, like Tc1 cells, Tc17 cells were TCF-1hi and KLRG-1lo indicating their capacity for long-term survival [35**,37**]. In view of the growing evidence for IL-17 producing cells in anti-fungal immunity [1,2,6**], it may not be surprising that Tc17 cells can mediate control of fungal infections in immune deficient hosts.

**Memory CD8+ T cell immunity:** Immunological memory is the hallmark of vaccination. Pathogen-specific memory T and B cells can persist in the absence of immunogen and are able to mount rapid anamnestic responses and clear infection following re-exposure, thus explaining the effect of vaccination. Since many fungal infections occur in the setting of immune deficiency where CD4+ T cells may be absent, reduced, or dysfunctional, it becomes crucial to understand the limits of memory maintenance under these unusual circumstances.

A typical CD8+ T cell response to an immunogen involves differentiation and rapid proliferation during an expansion phase, apoptosis of ~90% effectors during a contraction phase, and differentiation of the remaining ~10% of memory precursors into long-term memory cells during the memory phase. CD4+ T cells have been shown to foster the normal memory homeostasis of CD8+ T cells in models of viral and bacterial infection. By contrast, models of fungal infection have suggested that CD4+ T cells may not be required for generating and maintaining antifungal CD8+ T cell immunity. In a model of vaccine immunity against blastomycosis [37**], anti-fungal memory CD8+ T cells were generated in the absence of CD4+ T-cell help, and maintained without reduced quantity or function in the absence of the antigen, suggesting a unique priming environment that facilitates their long-term survival, and a distinct effector cell profile that is different from that following anti-viral or anti-bacterial immunization.

Why might anti-fungal CD8+ T cell responses be different? First, the models used have included live attenuated fungal strains, which may generate a unique inflammatory milieu that is different from subunit/recombinant/DNA vaccines. Second, whole cell-based vaccines promote cross-presentation, which, along with an appropriate inflammatory milieu, can give rise to a broad repertoire of CD8+ T cell responses. Third, this broad repertoire of CD8+ T cell responses may be less prone to exhaustion or
terminal differentiation. Fourth, CD8+ T cell responses in these models arise in a setting that gives rise to multifunctional cytokine profiles, including the production of IL-2, which is known to promote and help memory cell homeostasis. Fifth, the ligands that are present in whole cell-based fungal vaccines have been associated with a molecular signature that portends memory cell homeostasis. Thus, for individual fungal infections, understanding the mechanisms of immunity and the requirement for multiple signaling pathways engaged by whole cell vaccines will pave the way to robust immunization strategies or underscore the need for suitable adjuvants that target these pathways.

However, it is possible that eliciting memory responses against eukaryotic pathogens like fungi may be fundamentally different than that for viruses and bacteria. The existence of memory Th17 has been documented in mice and humans, but the long-term survival of bona fide Th17 cells has been debated [39, 40, 41, 42]. In a murine model of vaccine-induced immunity to blastomycosis in CD4-deficient hosts, anti-fungal effector Tc17 cells persisted over 2 months and portrayed a molecular signature that portended their long-term survival, albeit their long-term fate and plasticity remain to be determined [35**]. Unlike memory Th1 and Tc1 cells whose homeostasis is chiefly regulated by the cytokines IL-7 and IL-15, the requirements for Th17/Tc17 memory homeostasis might be complex and regulated differently [41].

Conclusions

Anti-fungal memory CD8+ cells have been elicited mainly with experimental vaccines that are comprised of live attenuated strains, which are feasible in the patients with suppressed immunity, including CD4+ lymphopenia. Although subunit recombinant vaccines are preferable in these circumstances, few if any suitable fungal antigens have been identified for CD8+ T cells. Until protective CD8+ T cell antigens are identified, it may be worth considering whole cell-based vaccines such as with the nonreplicating sporozoite vaccine against malaria [43**]. However, strategies for bolstering the long-term maintenance of protective immunity will need to be developed using killed or inactivated fungi [44].

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:
- of special interest
- of outstanding interest

5. This study gives the evidence that Th17 cells participate in fungal control during experimental oropharyngeal candidiasis.
7. This study illustrates that Th17 cells are necessary and sufficient to mediate vaccine-induced protection against 3 systemic mycoses.
14. This study provides evidence for the existence of human T cells against Cfl1 protein that mediate protection against multiple, distantly related pathogenic fungi.
This study gives evidence of the requirement for Tc17 cells in vaccine immunity and further implies their propensity for long-term memory.


This study uses rigorous methods to demonstrate the dispensability of CD4+ T cell help for the maintenance of protective antifungal CD8+ T cells.


This is the first report that TH17 may not become memory cells.


This is the first evidence that TH17 cells bear the features of stem-cells and survive as memory cells even though they converted into Th1 cells.


This study gives evidence that IL-23 signaling is required for memory TH17 responses.


The report of success of a whole cell-based vaccine against malaria, which is given intravenously.


This report establishes the surprising observation of cross protection against Candida and Staph aureus mediated by vaccination with ALS3, and defines the contributions of T cells, especially those elaborating IL-17.


This study demonstrates how sensing of fungal RNA by cross presenting DCs is required to elicit effective memory CD8+ T cell responses against Aspergillus.


This report evidences of CD8+ T-cell epitope of aspergillus showing potential protection against lethal pneumonia.


The study demonstrates the potential of T-cell based immunotherapy for invasive aspergillosis using defined peptides/proteins.


This study describes the role of CD8+ T cells during oropharyngeal candidiasis in humans.