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Harnessing the Immune Response to Fungal Pathogens for Vaccine Development

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Abstract

Invasive fungal infections are emerging diseases that kill over 1.5 million people per year worldwide. With the increase of immunocompromised populations, the incidence of invasive fungal infections is expected to continue to rise. Vaccines for viral and bacterial infectious diseases have had a transformative impact on human health worldwide. However, no fungal vaccines are currently in clinical use. Recently, interest in fungal vaccines has grown significantly. One *Candida* vaccine has completed phase 2 clinical trials, and research on vaccines against coccidioidomycosis continues to advance. Additionally, multiple groups have discovered various *Cryptococcus* mutant strains that promote protective responses to subsequent challenge in mouse models. There has also been progress in antibody-mediated fungal vaccines. In this review, we highlight recent fungal vaccine research progress, outline the wealth of data generated, and summarize current research for both fungal biology and immunology studies relevant to fungal vaccine development. We also review technological advancements in vaccine development and highlight the future prospects of a human vaccine against invasive fungal infections.

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Contents

THE HISTORICAL IMPORTANCE OF VACCINES

Vaccines are considered one of the most significant achievements in public health in human history. The importance of vaccines cannot be overstated. As Stanley Plotkin wrote in his book *Vaccines*, "The impact of vaccination on the health of the world's peoples is hard to exaggerate. With the exception of safe water, no other modality has had such a major effect on mortality reduction and population growth" (95). The development of vaccines against viral and bacterial infections has been highly successful. Although the idea of vaccination may have been developed much earlier, the term vaccine was first used by Edward Jenner in 1796 when he treated a young boy by inoculating him with the pus from a cowpox blister that contained vaccinia virus. The first smallpox vaccine was subsequently developed in 1798, and its mass use led to the eradication of smallpox almost two centuries later in 1979. A century after Edward Jenner's experiment, Louis Pasteur spearheaded the development of a live attenuated cholera vaccine and an inactivated anthrax vaccine for use in humans. Around the same time, a vaccine against *Yersinia pestis*, the cause of plague, was invented. Between 1890 and 1950, bacterial vaccine development expanded greatly, including the BCG (bacillus Calmette-Guérin) vaccine for preventing *Mycobacterium tuberculosis* infection. The availability of viral tissue culture methods in the 1950s led to the development of polio vaccines, which helped eradicate polio from many regions around the world (95). Vaccine development has now enabled the containment of a number of once highly prevalent infectious diseases in the United States, including polio, flu, hepatitis A, rubella (three-day measles), Hib (*Haemophilus influenzae* type B), measles, whooping cough, pneumococcal pneumonia, rotavirus infection, mumps, chickenpox, diphtheria, etc. An outbreak of a SARS-CoV-2 in late 2019 has caused a global pandemic of COVID-19. The urgency to control this ongoing pandemic has led to successful development of vaccines in record time, which is redefining vaccine development procedures.

However, despite these enormous successes, fungal vaccines have remained significantly underdeveloped. No fungal vaccines are available for clinical use in humans, and due to increases in chronic diseases and immunodeficiencies, invasive fungal infections have become more prevalent and more deadly. This has led to increased public awareness about the severity of these diseases and the importance of fungal vaccines. However, advances in our collective knowledge of fungal biology, fungal pathogenesis, and fungal immunology are required for breakthroughs in fungal vaccine development. Exciting new research and developments may finally help break the barrier

for a successful fungal vaccine. In this review, we summarize the current understanding of host immunity against fungal infections and its role in vaccine development.

THE NEED FOR FUNGAL VACCINES

The fungal kingdom contains over 1.5 million known species, and likely many more (42). Among these, several hundred fungal species have been reported to cause infections in humans and animals. Some fungal species cause invasive mycoses that are often life-threatening. Although some invasive fungal infections occur in healthy people, most infections occur opportunistically in immunocompromised hosts. Numerous factors have contributed to a dramatic increase in immunocompromised populations, including the HIV/AIDS epidemic, organ transplantations, other therapies that suppress immune function, increased life span due to the advancement of modern medicine, and improved living conditions. This demographic change has led to an increased risk for invasive fungal infections in immunocompromised populations, often with deadly consequences. Therefore, invasive fungal infections have become a significant public health concern, and current antifungal drugs are inadequate to effectively treat fungal infections. Because fungi are eukaryotic organisms, they share many cellular mechanisms with their mammalian hosts. Thus, developing suitable drug targets has proved difficult, with only three major drug classes currently available: polyenes (e.g., amphotericin B), azoles (e.g., fluconazole), and echinocandins (e.g., caspofungin). 5-Fluorocytosine, which targets DNA and RNA synthesis, has been used in combinational therapies together with amphotericin B to treat severe candidiasis and cryptococcal meningitis. While all these drugs have been successfully utilized for treatment of some fungal infections, several factors such as drug toxicity, high cost, and restrictions on administration methods often prevent timely access for many patients in critical need of them. In addition, the development of resistance to some antifungals has become a factor, and new species such as *Candida auris* have emerged that are highly resistant to our current arsenal of antifungals. Therefore, in addition to development of new antifungal drugs, fungal vaccines are highly desired and will likely significantly improve the well-being of immunocompromised populations who are at high risk, especially in regions with underdeveloped health care systems. The reasons for the lack of fungal vaccine development are complicated and include the complexity of fungal cell surface structures, an inadequate understanding of fungal immunology, and the fact that the majority of susceptible hosts are immunocompromised. However, recent advances in fungal biology and immunology have provided a much-improved understanding of invasive fungal infections.

IMMUNE MECHANISMS OF VACCINE-INDUCED PROTECTION AGAINST FUNGAL INFECTIONS

The fact that the majority of invasive fungal infections occur in individuals with specific immune deficiencies, such as HIV/AIDS patients, suggests host immunity plays a critical role in controlling fungal infections. Furthermore, due to much of the fungal-infection-susceptible population being immunocompromised, developing a vaccine for this population could be quite challenging. A better understanding of host immunity against fungal infections and how a vaccine candidate harnesses the host immune system could provide critical information. Recent advancements in fungal immunology and encouraging results in vaccine-induced protection in immunocompromised animal hosts have provided much-needed support for fungal vaccine development.

Adaptive Immunity

Protection against a variety of fungal infections strongly depends on effective host T cell responses (34, 36, 78). There is extensive evidence for the critical role of $CD4^+$ T cells in protection against

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multiple clinically important fungal pathogens, including *Candida*, *Pneumocystis*, *Blastomyces*, *Histoplasma*, *Coccidioides*, and *Cryptococcus* (112, 135, 145). Animal models of infection show that removal of $CD4^+$ T cells results in enhanced susceptibility to fungal infection and is linked to an impaired control of fungal cells at the infection site (31, 44, 50, 51, 58, 109, 110, 131). Clinically, patients with reduced $CD4^+$ T cell counts are at increased risk of developing deadly fungal infections (68, 76, 105, 111). In fact, at the height of the HIV epidemic, fungal infections, including *Pneumocystis* and *Cryptococcus* infections, were AIDS-defining illnesses (19, 91, 94). To date, opportunistic fungal infections continue to be a significant cause of AIDS-related mortality (4, 71, 122). Given the importance of $CD4^+$ T cells in orchestrating protective immunity, it is not surprising that vaccine-induced protection in various models of fungal infection has been shown to depend on appropriate activation of T cell responses (29, 81, 130, 152). Activation and differentiation of IFN-γ-producing Th1 cells is also critical in vaccine-mediated protection against multiple fungal pathogens (3). Th1 cell–derived cytokines activate phagocyte responses that help contain and eliminate fungal cells (6, 57, 80, 153). Th17 cells have also emerged as critical mediators of defense against diverse fungal infection (1, 21, 43, 113, 139). Th17-derived cytokines, IL-17 and IL-22, promote the recruitment of neutrophils, critical innate immune cells that contribute to the eradication of multiple fungal pathogens (21, 53). In addition, Th17 responses promote the secretion of antimicrobial peptides that can restrain fungal cells (65). Thus, it is not surprising that vaccine-induced fungal protection has also been linked to activation of Th17 responses, with potent protection often being elicited by the coordinated activation of both Th1 and Th17 responses (82, 147).

Given the high susceptibility of $CD4^+$ T cell–deficient patients to fungal infections, effective vaccines against fungal pathogens would ideally confer protection in the setting of $CD4^+$ T cell deficiency. Various animal models have provided promising evidence for the possibility of eliciting $CD4^+$ T cell–independent protection against multiple fungal infections (25, 67, 142, 151). In a model of blastomycosis, $CD8^+$ T cells were found to compensate for the absence of $CD4^+$ T cells (83). Similarly, several candidate vaccine strains confer protection against cryptococcosis in $CD4^+$ T cell–depleted mice (87, 98, 138, 142). Mechanistically, it has been proposed that in the absence of $CD4^+$ T cells, $CD8^+$ T cells can expand and serve as a critical cytokine source, especially of IFN-γ and occasionally also IL-17 (66, 67, 80). Thus, emerging evidence suggests the possibility of developing fungal vaccine formulations that can promote broad activation of host immunity in a manner that can still protect in settings of selected immune dysfunction. It remains unclear whether similar vaccine-induced antifungal immunity mechanisms can operate in patients living with HIV. Important factors to consider include the restoration of stable $CD4^+$ T cell levels in HIV-positive patients receiving highly active antiretroviral therapy (HAART) that may allow for effective vaccination (22). Additionally, the timing of vaccination may allow for effective immunization in patients treated during early stages of HIV infection (38). Clinical recommendations stress the importance of vaccinations in HIV-infected individuals, and studies show activation of protective responses following immunization to influenza, hepatitis B virus and pneumococcus (8, 33). Thus, fungal vaccines could be selected for their ability to stimulate CD4-independent protection in HIV patients with reduced $CD4^+$ T cell counts. Another possible approach is the use of vaccines that mediate protection via generation of antibodies or development of monoclonal antibodies to boost fungal growth control (reviewed in 11, 132).

An exciting aspect of preclinical studies in fungal vaccines has been the identification of conserved epitopes that provide protection across diverse fungal pathogens (128). Given the complex nature of fungal pathogens and the various challenges faced in the effective implementation of antifungal vaccination programs, the development of a universal vaccine against fungi would overcome many of these challenges (100). Proof-of-concept studies have demonstrated the presence

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of conserved, protective T cell epitopes across *Ascomycetes* (143). Calnexin-specific T cells were uncovered to mediate protection against diverse dimorphic fungi (143). Calnexin-specific T cells were also activated in response to *Aspergillus* and dermatophytes (143). Thus, it may be possible to design vaccine formulations that confer broad protection against multiple clinically relevant fungal pathogens. The identification of the fungal antigen Engl2 as a molecule with combined features of adjuvant and T cell epitopes is another intriguing proof-of concept finding that could guide studies of various fungal pathogens and help identify molecules with similar features (137).

Various studies support inclusion of fungus-derived pathogen-associated molecular patterns (PAMPs) as potent adjuvants that can help tailor vaccine-induced immune responses (**Figure 1**). Members of the C-type lectin family of receptors have emerged as crucial activators of antifungal innate immune responses (40, 41). Important roles for Dectin-1, Dectin-2, and Mincle have been identified, not only as activators of innate effector functions but also as regulators of T cell differentiation toward protective Th1 and Th17 lineages (114). Fungal vaccine formulations that include combinations of fungal PAMPs have shown great promise in mouse models as mediators of tailored antifungal immunity. Among these, glucan particles, which are enriched with β-glucans and chitin, can be loaded with various antigens and used as a delivery platform for antifungal vaccines (discussed in more detail in sections below). The use of glucan particles facilitates delivery of relevant fungus-derived antigens together with adjuvants optimized for programming antifungal defenses. In this setting, optimal vaccine-induced protection was mediated by Th1 and Th17 cells and involved engagement of host innate immune receptors Dectin-1 and Dectin-2 and the downstream signaling partner CARD-9.

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Innate Cells and Trained Immunity

The potent adaptive immune responses that need to be elicited by antifungal vaccines are critically influenced by proper activation of innate immune cells and, in particular, dendritic cells that help shape T cell differentiation. C-type lectins are critical innate receptors that shape Th1 and Th17 cell differentiation via effects on innate immune cells and promote cytokine secretion that supports antifungal immunity (40). Toll-like receptors, especially TLR2, TLR4, and TLR9, are also involved in the activation of antifungal responses and vaccine-induced protection (5). Thus, vaccine formulations that include adjuvants to engage these pathways have shown promise in various studies. In classic vaccination strategies, the primary role of innate cells has been understood to be coordination of adaptive immune cells, which then act as the ultimate effectors of protection. Exciting developments in recent years have expanded the role for innate cells in vaccine-induced protection, as well as their ability to retain memory features originally associated with only T and B lymphocytes. The most accepted term for these features is trained immunity (30, 86). Seminal studies have shown that innate cells can undergo innate training and be programmed with long-term changes that promote enhanced responses upon secondary challenges (30). In these studies, fungal PAMPs were critical mediators of trained immunity (**Figure 2**). Murine studies show prior immunization with heterologous vaccines such as BCG (bacillus Calmette-Guérin) vaccine can confer protection against candidiasis in a T cell–independent manner (17). Importantly, heterologous protection involved the engagement of C-type lectins. Moreover, ex vivo studies showed β-glucan recognition (via Dectin-1 on monocytes) induced trained immunity in monocytes through HIF-α signals that subsequently induced epigenetic changes (17). These findings suggest that mechanistically, fungus-derived PAMPs can help program epigenetic changes in innate immune cells and thus endow innate effectors with enhanced responses to fungal infections. Importantly, trained innate cell immunity has the critical feature of promoting protection not only against the priming antigen but also against

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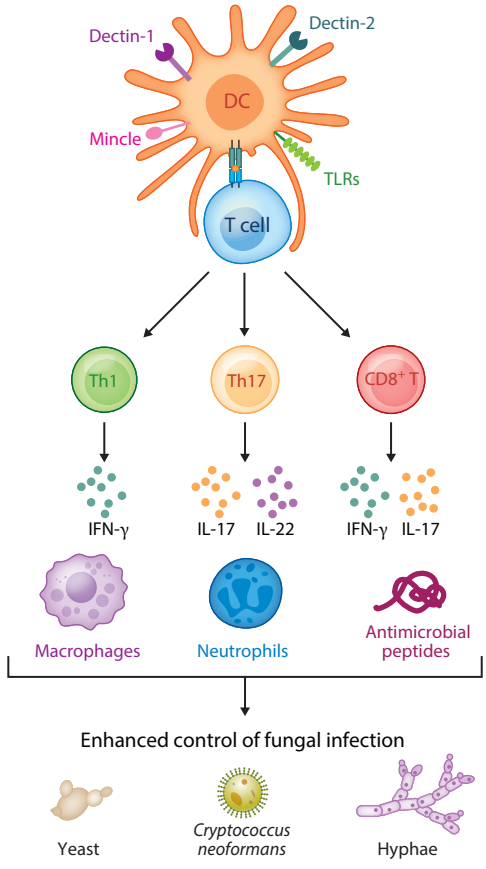

Figure 1

Overview of adaptive immunity. Innate cells recognize molecular patterns present in fungal pathogens via innate receptors. The C-type lectins Dectin-1, Dectin-2, and Mincle have been identified as critical activators of host immune responses in the context of fungal infection. Toll-like receptors (TLRs) are also engaged during fungal infection and together with C-type lectins help activate dendritic cells (DCs). In turn, DCs are critical for the activation of T cell responses via presentation of fungus-derived antigens in the context of MHC together with proper costimulation and secretion of cytokines that shape T cell differentiation. The differentiation of $CD4^+$ T cells toward Th1 and Th17 cells is critical for antifungal defense and the activation of effective vaccine-mediated immunity. Th1 and Th17 cells produce critical cytokines, such as IFN-γ, IL-17, and IL-22, that act on innate cell effectors to further amplify the effective control of fungal infection. Effector cytokines also help induce the expression of antimicrobial peptides that can have direct toxic effects on fungal cells. Effector cytokines also act on innate cell targets. These include macrophages and neutrophils, which are significantly involved in direct eradiation of various fungal pathogens and morphotypes that range from yeast to hyphal forms. The activation of robust antifungal immunity also involves $CD8^+$ T cells, which can also serve as an important source of protective cytokines. $CD8^+$ T cells are particularly important in the context of $CD4^+$ T cell deficiency, where an expansion of $CD8^+$ T cells can compensate for loss of $CD4^+$ T cells and help mediate vaccine-induced protection.

heterologous infections. Thus, future studies in trained immunity promise to provide the basis for tailored designs of potent antifungal vaccines capable of exploiting both innate and adaptive immune cells and providing broad protection against fungal pathogens. The potential detrimental impact of trained immunity via activation of inflammatory pathology remains to be fully explored, but there are indications that this is an important factor to be considered in future studies (85).

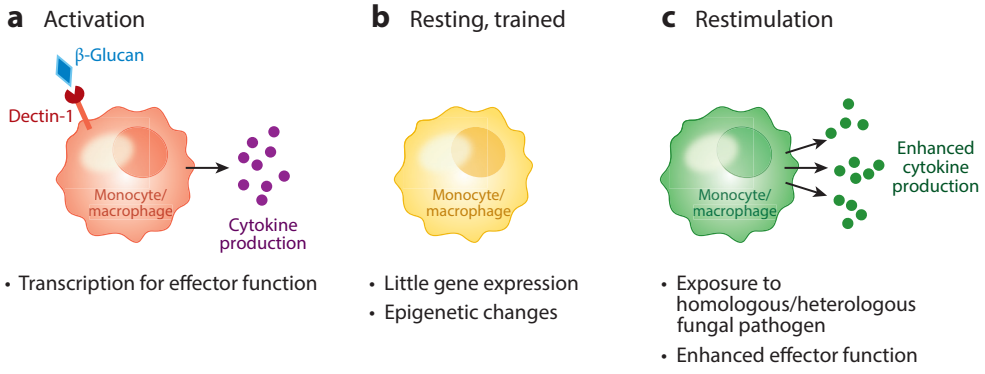

Figure 2

Overview of trained immunity. In the context of fungal infection, vaccination with formulations that include fungus-derived adjuvants such as β-glucan can mediate the programing of monocytes and macrophages for enhanced secondary responses, known as trained immunity. (*a*) Recognition of β-glucan via Dectin-1, for example, has been found to activate innate cells for increased transcription, cytokine secretion, and effector functions during initial encounter with fungi. (*b*) This primary response also triggers epigenetic changes in the now experienced cells and leads to alterations in their chromatin structure such that they are poised for rapid and robust production of cytokines during a secondary insult. (*c*) Importantly, enhanced secondary responses in trained innate cells not only help mediate homologous protection but also can be potent inducers of heterologous defense.

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CHALLENGES TO DEVELOPING FUNGAL VACCINES

Despite rapid advances in fungal immunology and the identification of many immunogenic mutant strains and fungal factors that are antigenic in animal models, a very limited number of vaccine candidates have been further developed and tested in human clinical trials. Several complex factors likely contribute to the slow progress toward important fungal vaccine development and commercialization. As immunodeficiency is a major risk factor for fungal infections, an ideal fungal vaccine would be effective in immunocompromised hosts. This efficacy is required for targeting large immunocompromised populations and having a significant impact on public health. Although many cases of fungal infection have been reported in healthy people and some fungal pathogens are primary pathogens, the fungus-infected population may not be large enough to attract industry interest, especially if vaccines only work in individuals with normal immunity. Furthermore, identifying at-risk populations could prove challenging. Recently, a number of vaccine candidates have been effective in immunocompromised hosts. Multiple *Cryptococcus* vaccine candidates have successfully immunized mice with continued protection against *Cryptococcus*, including in $CD4^+$ T cell–depleted mice. Additionally, mice vaccinated with heat-killed *fbp1*Δ mutant (HK-fbp1) cells and treated with the immunosuppression drug cyclophosphamide remained protected against *Aspergillus fumigatus* (138). Both *Cryptococcus* H99γ and *sgl1*Δ vaccine strains are effective in $CD4^+$ T cell– or $CD8^+$ T cell–depleted mice, but not in dually depleted mice (87, 142). These promising developments suggest it is possible to develop vaccines against fungal infections, even in immunocompromised hosts.

Another factor limiting fungal vaccine development is the number of different fungal pathogens that can cause infections. Although collectively fungal infections constitute a major public health burden on a global scale, individual fungal pathogens typically impact specific populations in geographically restricted areas. Additionally, people susceptible to fungal infection, e.g., those with HIV/AIDS, are often at risk for infections by multiple fungal pathogens. Ideally, a

pan-fungal vaccine capable of protecting at-risk populations from infections by multiple fungal pathogens would significantly increase the impact on public health and attract the interest of the pharmaceutical industry. A few studies have reported vaccine candidates capable of protecting immunized animals against multiple invasive fungal pathogens. For example, HK-fbp1-vaccinated mice are protected against *Cryptococcus neoformans*, *Cryptococcus gattii*, and *A. fumigatus* (138), while mice immunized with the rAls3p-N vaccine (NDV-3A) are also protected against the bacterial pathogen *Staphylococcus aureus*.

Fungal cells have complex cell wall structures, which also increases the difficulty of identifying antigenic factors for vaccine development. Antigenic factors will not necessarily be a single component in many whole cell–based vaccine candidates. It may also be challenging to identify antigenic factors and optimize the vaccine, both of which would increase vaccine development costs and affect potential efficacy.

The limitations described above may have an impact on the size of the market for fungal vaccines, as well as the interest of the pharmaceutical industry and private funders. Funding sources beyond industry partners, such as government agencies and foundations, may provide support to bridge funding gaps for fungal vaccine development.

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STATUS OF FUNGAL VACCINE RESEARCH AND DEVELOPMENT

In recent years, rapid advances in fungal immunology and fungal gene functional analyses have identified several mutant fungal strains capable of inducing Th1 and Th17 protective immunity. These strains also impart protection against virulent fungal strains in animal models. These findings have sparked new research interest in fungal vaccine development. Although many vaccine candidates have been proposed or reported for a variety of fungal pathogens, as summarized in several excellent review articles (3, 7, 23, 59, 79, 88, 118) (**Table 1**), more systematic research has also been done on several well-studied fungal infections. These thorough studies have led to a better understanding of fungal biology and immunology. Here, we provide a broad summary but also focus on a handful of major fungal pathogens.

Candida Vaccine Candidates

Candidiasis, typically caused by *Candida albicans*, is the most common yeast infection in humans and the fourth-most common bloodstream infection in hospitalized patients in the United States (140). *C. albicans* is a commensal human gut microbiota species. In immunocompromised or other impaired host conditions, *Candida* can undergo morphological changes to produce infectious pseudohyphae and hyphae. Successful infection by *C. albicans* relies on multiple virulence strategies, including high plasticity in morphological switching and robust biofilm formation (124). *C. albicans* then disseminates through the bloodstream to cause systemic infection. While *C. albicans* is the most common species causing candidiasis, several other *Candida* species can cause invasive candidiasis, including *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*. Due to the increased use of antifungal drugs, some *Candida* species are increasingly drug resistant. Furthermore, certain drug-resistant species, such as *C. glabrata*, have become more abundant in certain areas, such as Europe (136). The recent outbreak of *C. auris* in hospital settings has raised significant concern, as this species is often resistant to multiple antifungal drug classes. Due to the limited number of antifungal drugs, increased drug resistance, and scarce antifungal drug availability in large parts of the world, non-drug-based treatment strategies are urgently needed. Ideally, an effective vaccine against *Candida* infection would also overcome the increasing challenges of antifungal drug resistance.

Table 1 Fungal vaccine candidates

Vaccine	Background	Mechanism	Target diseases	Status	Reference(s)
Whole-cell vaccines (live attenuated or killed cells)					
PCA-2	Live *Candida albicans* strain lacking yeast-hypha conversion	Increased polymorphonuclear cells and macrophage activities	Candidiasis *Staphylococcus aureus* infection	Murine model	2
CNC13	Live *C. albicans* strain deleted of kinase Hog1	Immunoglobulins and IgG2a	Candidiasis	Murine model	35
Ter-NRG1	Live *C. albicans* overexpressing transcription factor Nrg1	T cell response	Candidiasis	Murine model	108
RML2U	Live *C. albicans* strain deleted of Ecm33	Antibody response	Candidiasis	Murine model	74
cph1/efg1	Live *C. albicans* strain deleted of transcription factors Cph1 and Efg1	Not defined	Candidiasis	Murine model	149
H99γ	Live *Cryptococcus neoformans* strain expressing human IFN-γ	T cell response	Cryptococcosis	Murine model	141
Cda123	Live or heat-killed *C. neoformans* strain lacking chitin deacetylase activity	T cell response	Cryptococcosis	Murine model	134
Znf2OE	*C. neoformans* strain overexpressing transcription factor Znf2	T cell response	Cryptococcosis	Murine model	150
HK-fbp1	Heat-killed *C. neoformans* strain deleted of Fbp1	T cell response	Cryptococcosis Aspergillosis	Murine model	75, 138
Sgl1	Live *C. neoformans* strain deleted of Sgl1	T cell response	Cryptococcosis	Murine model	20
Killed spherules	Formalin-killed *Coccidioides immitis* strain	Not defined	Coccidioidomycosis	Human phase 3 trial	90
Cps1	Live *C. immitis* strain deleted of Cps1	T cell response	Coccidioidomycosis	Murine model	84
Bad1	Live *Blastomyces dermatitis* strain deleted of Bad1	Multiple arms of immune responses	Blastomycosis	Murine model	144
Recombinant or subunit vaccines					
NDV-3A	Agglutinin-like adhesion protein Als3, with Alhydrogel adjuvant	T cell– and antibody-mediated responses	Candidiasis *S. aureus* infection	Human phase 2a trial	32, 119
PEV7	Secreted aspartyl protease Sap, with cholera toxin adjuvant	Antibody response	Candidiasis	Human phase 1 trial	24
CWSP	β-Mercaptoethanol-extracted *Candida* cell wall proteins, with liposomal adjuvant	Antibody and Th17 responses	Candidiasis	Murine model	126
Mannan-glycopeptides	*C. albicans* glycopeptides conjugated with β-mannan	Antibody response	Candidiasis	Murine model	148

(*Continued*)

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Table 1 (*Continued*)

Vaccine	Background	Mechanism	Target diseases	Status	Reference(s)
β-Glucan-CRM197	*C. albicans* β-glucan-CRM197 conjugates, with complete Freund's adjuvant	Antibody response	Candidiasis	Murine model	37
GXM-TT	*C. neoformans* GXM conjugated with tetanus toxoid	Antibody response	Cryptococcosis	Murine model	37
GXM-BSA	*C. neoformans* GXM conjugated with BSA	Antibody response	Cryptococcosis	Murine model	18
Glucan particles	Glucan particles packaged with *C. neoformans* alkaline extracts	Antibody and T cell responses	Cryptococcosis	Murine model	115, 116
Aspf2	Recombinant allergen; only works in immuno-competent hosts	T cell response	Aspergillosis	Murine model	13
Crf1	Cell wall glucanase Crf1, with CpG adjuvant	T cell response	Aspergillosis Candidiasis	Murine model	123
AF.KEX1	Protease KexB; protects immunocompromised mice	Antibody response	Aspergillosis	Murine model	97
Antigen 2	Proline-rich antigen on cell surface, with CpG adjuvant	T cell response	Coccidioidomycosis	Murine model	56
rHsp60	*Histoplasmosis capsulatum* recombinant glycoprotein Hsp60	T cell response	Histoplasmosis	Murine model	39
H antigen	Recombinant β-glucosidase protein in *H. capsulatum*	T cell response	Histoplasmosis	Murine model	27
Antibody-based vaccines					
Mycograb	*C. albicans* Hsp90 recombinant antibody	Therapeutic antibody	Candidiasis	Human trials	89
GXM antibody 18B7	*C. neoformans* GXM monoclonal antibody	Therapeutic antibody	Cryptococcosis	Human phase 1 trial	62
P13	A peptide mimic of *C. neoformans* GXM	Antibody response	Cryptococcosis	Murine model	37
Anti-Crf1	Neutralizing anybody against Crf1 protein	Therapeutic antibody	Aspergillosis	Murine model	15
β-Glucan antibody	β-Glucan monoclonal antibody	Therapeutic antibody	Cryptococcosis	Murine model	96
Glucosylceramide antibody	Monoclonal antibody	Therapeutic antibody	Cryptococcosis	Murine model	104

Abbreviations: BSA, bovine serum albumin; GXM, glucuronoxylomannan; Hsp60, heat shock protein 60; rHsp60, recombinant Hsp60; TT, tetanus toxoid.

Over the years, multiple fungal vaccine candidates have shown efficacy in animal models (**Table 1**); however, only a few have been tested in human clinical trials. Several *C. albicans* mutant strains have shown efficacy in animal models and have been proposed for human vaccine development. As the yeast-to-hyphae transition is a major virulence factor in *C. albicans*, proteins required for such a dimorphic switch have been characterized for their role in host-pathogen interaction

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and infection. Several mutants defective in the yeast-to-hyphae transition have been shown to induce protective immunity in mice against wild-type *C. albicans* challenge. Among these hyphal defective strains are the PCA-2 strain that is resistant to echinocandins and agerminative (2), the CNC13 strain that lacks the MAP kinase Hog1 protein (35), the NRG1 strain that overexpresses the filamentation repressor Nrg1 (108), the RML2U strain that lacks the cell wall protein Ecm33 (74), and the *cph1/efg1* double mutant strain that locks fungal cells in yeast phase (149). These mutants have all been tested as vaccines to protect against challenge by their respective wild-type strains, and shown positive outcomes in animal models. In addition, the PCA-2 vaccine also shows cross protection against *S. aureus*, suggesting a conserved antigenic factor in these organisms or nonspecific host immune activation mechanisms that remain poorly understood. Despite positive protection in murine models, none of these candidates has been tested in human clinical trials. Lack of development for human trials may be due to the effect of most of these fungal infections on immunocompromised hosts. Use of live attenuated vaccines in immunocompromised hosts has clear safety concerns, including the chance of the host developing disease. *C. albicans* is a commensal human gut organism, and a minor concern also exists that a *Candida* vaccine may affect the normal host gut microbiota.

Beyond using live attenuated vaccines based on virulence-defective mutants, exciting developments have been reported for recombinant-protein-based approaches. These approaches have mainly focused on the Al (agglutinin-like sequence) and Sap (secreted aspartyl protease) protein families. Als are fungal surface glycoproteins that play an important role in yeast adhesion and are required for *Candida* infection (46). This eight-member protein family shares a four-domain structure consisting of (*a*) a high-complexity N-terminal domain that mediates interaction with host cells or other substrates, (*b*) a threonine-rich domain, (*c*) a central domain, and (*d*) a C-terminal domain that anchors the protein to the fungal cell wall through a glycosylphosphatidylinositol anchor (46). Following the first report of an α-agglutinin-like protein in 1998 (47), studies on Al gene family members have provided a deep understanding of their functions, especially their role in *Candida* adhesion to host surfaces and medical implants. Among the eight Al proteins in *C. albicans*, Als1 and Als3 have been extensively studied in genetic and biochemical analyses to understand their roles in adhesion, biofilm formation, and fungal virulence (16, 54, 92, 121). Since both of these Als proteins are expressed on the fungal surface and are important for virulence, recombinant Als1 and Als3 protein fragment vaccine approaches have been proposed. The N termini of Als1 (rAls1p-N) and Als3 (rAls3p-N) have both been successfully utilized for vaccine development with or without adjuvants (54, 70, 121). rAls1p-N was expressed and purified in *Saccharomyces cerevisiae* and was used in combination with complete Freund's adjuvant, being administrated subcutaneously. A booster was given at 21 days after immunization, before challenge with wild-type *C. albicans*. This vaccine approach was deemed effective, reducing fungal burden and displaying an improved murine survival rate of 50–57%. The vaccine also protected neutropenic mice from oropharyngeal candidiasis as well as vaginal candidiasis (54, 121). Compared to rAls1p-N, the rAls3p-N recombinant vaccine shows an even stronger antibody response and improved animal survival in murine models (120). The rAls3p-N vaccine, based on the N terminus of Als3, also protects mice against the bacterial pathogen *S. aureus* (119). This cross protection is likely due to the similarity of antigenic factor, as Als3 is structurally similar to a clumping factor in *S. aureus*. Encouragingly, an rAls3p-N vaccine formulated with an Alhydrogel adjuvant has been tested in women in a phase 1b/2a clinical trial for recurrent vulvovaginal candidiasis (RVVC), with promising results (32).

The ten-member Sap gene family represents a new virulence factor that is required for *Candida* virulence through regulation of the yeast-to-hyphae transition, as well as for host adhesion and deep-tissue penetration of the fungus (49, 61). Among this family, Sap2 is most

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abundant in *C. albicans*. A vaccine based on recombinant Sap2 (rSap2) is capable of clearing vaginal candidiasis in animal models. PEV7, a modified version of the rSap2 vaccine that consists of a truncated recombinant Sap2 incorporated into influenza virosomes, induces anti-Sap2 IgG and IgA. Furthermore, PEV7 induces long-lasting protection when administered as an intravaginal immunization (24). PEV7 was also safe in a repeated-dose toxicological study in rats. PEV7 is being tested in a phase 1 clinical trial for RVVC; the vaccine has yielded positive outcomes for all immunized patients and produced mucosal immune responses with high titers.

Mycograb, codeveloped by Novartis, is a human recombinant antibody against fungal Hsp90 that consists of Hsp90 peptide (NKILKVIRKNIVKK) cross-linked with human heavy-chain antibody. The heat shock protein Hsp90 is a highly conserved chaperone protein on fungal cell walls that is induced by stress stimuli. Hsp90 is abundant and immunogenic and is required for cell viability in *C. albicans* (77). In combination with amphotericin B, Mycograb significantly improves survival in a murine model of systemic candidiasis (89). However, Mycograb did not gain regulatory approval and was later found that its ability to potentiate amphotericin B effects were likely not Hsp90-specific (99).

Cell wall extracts and some cell wall components, such as mannans and β-glucans, are conjugated with an antigenic factor or adjuvants that have been tested for potential vaccine development against *Candida* spp. Mice immunized with β-mercaptoethanol-extracted *C. albicans* cell wall protein mixtures in combination with the Ribi Adjuvant System (RAS) R-700 showed improved survival when challenged with *C. albicans* (126). Fungal glycoconjugates, such as mannans and β-glucans, are major PAMPs and can be recognized by pathogen recognition receptors. As such, they have been investigated as vaccine candidates. Cell surface mannans are among such PAMPs and are recognized by multiple host receptors. Consistent with the observation that mannosylated antigens demonstrate more effective presentation than nonmannosylated antigens, cell surface peptides linked with mannans have been utilized as vaccine candidates in murine models of *C. albicans* immunization (148). *C. albicans* β-glucan is another cell surface antigenic component that elicits Dectin-1 receptor–mediated innate immunity (125). Fungal β-glucan in combination with MF59 adjuvant or diphtheria toxoid (Lam-CRM197) has shown protection against murine vaginal candidiasis (93, 127). However, although highly immunogenic, the complex nature of mannans and β-glucan exposure on fungal cell surfaces may also result in varied host protection. Better mechanistic understanding of polysaccharide-mediated immune responses will likely be required before translation of these findings into safe and effective human antifungal vaccines (129).

Cryptococcus Vaccine Candidates

Cryptococcosis is a life-threatening fungal infection mostly caused by inhaling environmental fungal spores and yeasts produced by *C. neoformans* and *C. gattii*. Inhaled fungal cells lodge deep into lung alveoli and cause pulmonary infection. Cells often then disseminate to the central nervous system and cause cryptococcal meningoencephalitis, which is uniformly fatal without proper treatment (52). *Cryptococcus* infection is the leading cause of fungal meningitis and accounts for roughly 15% of HIV/AIDS-related deaths. Although *C. neoformans* is considered an opportunistic pathogen that often infects immunocompromised populations, *C. gattii* can infect immunocompetent individuals and is thus considered a primary pathogen. Similar to treatments for candidiasis and other invasive mycoses, cryptococcosis treatment is limited, and specific vaccines are needed. Although no vaccine is available, there are exciting ongoing research activities to develop prophylactic vaccines against cryptococcus infection.

The *C. neoformans* capsule has a unique structure and is a key virulence factor. The *C. neoformans* polysaccharide capsule comprises primarily glucuronoxylomannan (GXM) and galactoxylomannan, with a small amount of mannoproteins. This polysaccharide capsule enables cryptococcal

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cells to evade the host immune system and cause systemic infection. Early *C. neoformans* vaccine research used polysaccharide antigens. However, the polysaccharide capsule is generally considered a T cell–independent type 2 antigen, has anti-inflammation and antiphagocytosis functions, and is thought to suppress Th1 protective immunity—making capsule GXM a poor immunogenic antigen. Indeed, GXM-only vaccines do not induce the strong immune responses associated with long-term immune memory. To overcome this, polysaccharide-based vaccines were later constructed of capsule protein conjugated to other antigenic carriers, e.g., tetanus toxoid (TT) (28, 37) or bovine serum albumin (BSA) (18, 37). These GXM conjugated vaccines showed an improved animal survival rate after fungal challenge in murine models (10). Recently, a GXM monoclonal antibody, 18B7, has been developed as a potential neutralizing antibody. A phase 1 clinical trial of 18B7 in HIV patients with cryptococcal antigenemia has been completed. 18B7 displays a modest reduction in cryptococcal antigen titers (9, 62). However, this titer reduction is not long-lasting, suggesting further modifications may be necessary for a sustained response. Other monoclonal antibodies generated against *Cryptococcus* cell wall components such as β-glucan (96), glucosylceramide (104), and melanin (106) have also been utilized to passively immunize mice, with only modestly positive outcomes. However, all these antibodies did reduce lung fungal burden and prolong survival of treated mice.

In addition to capsule, other fungal antigenic factors have been identified, potentially providing opportunities for single-component-based vaccine development. Analysis of fungal antigen fractions revealed mannoprotein as an important antigenic component for stimulating cell-mediated immunity. These findings led to the identification of MP98, a chitin deacetylase motif required for converting chitin to chitosan, and MP88, whose function remains unknown (48, 63). Studies of these mannoproteins have demonstrated the importance of posttranslational modification in recombinant-protein-based vaccine design, as N- and O-linked mannosylation is essential for antigen recognition and optimal T cell responses (64, 117). However, not all mannoproteins display the same proinflammatory responses, and a more detailed understanding of individual mannoprotein fractions is required. Furthermore, the combination of certain adjuvants with mannoprotein antigens may be required to induce desired host immune responses (72). One fungus-based adjuvant is β-glucan particles (GPs), which have been developed as an antigen delivery platform. *Cryptococcus* alkaline extracts packaged in GPs have been tested as a vaccine strategy against cryptococcosis. Mice immunized with GPs containing fungal extracts and single-antigen proteins produced antigen-specific $CD4^+$ T cell recall responses (115, 116). Vaccinated mice also exhibited prolonged protection against *C. neoformans* or *C. gattii* challenge (115, 116). One exciting aspect of this system is that GPs act both as a delivery system and as an adjuvant. This property allows for a vaccine strategy where GPs are used to package different antigenic fractions and are mixed with different antigens into a single vaccine—increasing vaccine development feasibility and flexibility.

Recently, multiple groups have reported exciting findings toward the development of whole-cell-based vaccine candidates derived from genetically modified *Cryptococcus* strains. Given the importance of Th1 cytokine IFN-γ in anticryptococcal activity, *C. neoformans* wild-type strain H99 expressing murine IFN-γ (H99γ) was engineered and tested in a murine model of systemic cryptococcosis (141). Mice infected with live H99γ resolved their infection and showed full protection against subsequent challenge of H99, with complete clearance (141). Impressively, follow-up studies showed that protection remains even in mice depleted of $CD4^+$ or $CD8^+$ T cells by antibodies (142). Trained innate immunity was also proposed to be important in this H99γ-mediated vaccine protection (45). Several other genetically modified *Cryptococcus* strains have also shown strong protection in murine models against H99 challenge. These strains include a *Cryptococcus* strain locked in pseudohypha form due to overexpression of the mating-specific

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transcription factor Znf2 (Znf2OE) (150), a strain where chitin deacetylase genes (*cda1*Δ*2*Δ*3*Δ) are deleted (133, 134), a strain lacking sterol glucosidase (*sgl1*Δ) (20, 87, 98), and an *fbp1*Δ null mutant strain that lacks SCF E3 ligase subunit Fbp1 function (69, 75, 138). Vaccination with H99γ and *sgl1*Δ live cells, but not their heat-inactivated counterparts, conferred protection in mice. Furthermore, vaccination with heat-inactivated *cda1*Δ*2*Δ*3*Δ, Znf2OE, and *fbp1*Δ cells also conferred strong protection. Impressively, some of these vaccine candidates (H99γ, *cda1*Δ*2*Δ*3*Δ, *fbp1*Δ, *sgl1*Δ) conferred protection even in T cell–depleted mice, suggesting that they may be effective in immunocompromised patients with low $CD4^+$ T cell counts. Mice immunized with heat-killed *fbp1*Δ cells showed a broad spectrum of protection, with strong protection not only against *C. neoformans* and *C. gattii* challenge, but also against *A. fumigatus*, another major fungal pathogen. It will be interesting to determine whether this *fbp1*Δ vaccine contains a conserved antigenic factor or other immune mechanism that could trigger trained innate immunity. Although GXM secretion is important for *sgl1*Δ vaccine protection, the detailed mechanisms of protection in many of these strains, including *fbp1*Δ, remain elusive. In the future, it would be informative to compare these diverse vaccine strains to determine whether they share specific common core antigenic factors or immune mechanisms that allow them to confer protection. It will also be important to identify the specific factors that are sufficient to confer protection in each vaccine candidate. This would provide a better understanding of the unique protection mechanisms for each strain. Such studies may require joint efforts and greater collaboration among groups interested in *Cryptococcus* vaccine research and development.

Aspergillus Vaccine Candidates

A. fumigatus is a major filamentous fungal pathogen that causes significant invasive infection with a high mortality rate. As *A. fumigatus* infects mostly people with severe immunodeficiencies, including neutropenic patients, vaccine research and development has been limited. With an improved understanding of fungal immunology, we now understand some vaccines can be protective even in immunocompromised individuals. A prophylactic *Aspergillus* vaccine would still be very valuable for specific patient populations, such as individuals undergoing organ transplantation or cancer chemotherapy. Early studies showed that *A. fumigatus* crude culture filtrate antigens and the recombinant allergen Aspf2 can induce development of local and peripheral protective Th1 memory responses. This approach resulted in protective antifungal responses in mice with invasive pulmonary aspergillosis. However, this induction was only detected in immunocompetent mice and not IFN-γ- or IL-12-deficient immunocompromised mice (13). The complex nature of the cell extract mixture used in this approach may make it difficult to determine the specific mechanism of antigenicity and to produce vaccines on a large scale with consistent outcomes.

A vaccine candidate based on an immunogenic epitope of the *A. fumigatus* cell wall glucanase Crf1 has been studied quite extensively. The Crf1 epitope induces memory $CD4^+$ Th1 cells and elicits cross protection against lethal infection with both *A. fumigatus* and *C. albicans* (123). Recently, anti-Crf antibodies were found to neutralize the enzymatic activity of recombinant Crf1 protein, and when added to spores they led to delayed fungal growth in vitro. These studies demonstrate the therapeutic potential of targeting Crf cell wall proteins with anti-Crf antibodies (15). More recently, AF.KEX1, a recombinant vaccine candidate based on the *A. fumigatus* protease protein KexB, showed protection in immunocompromised mice that were treated with steroids or tacrolimus/hydrocortisone following challenge with wild-type *Aspergillus*. Vaccinated mice had prolonged survival rates and had reduced lung fungal burden compared to unvaccinated mice (97). However, despite these positive findings in animal models, no *Aspergillus* vaccine candidates have reached human trials.

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Vaccine Candidates Against Endemic Mycoses

In addition to the major global fungal pathogens described above, endemic fungi exist in certain geographic regions and occupy specific niches. These endemic fungi can cause systemic infection in healthy human populations. Due to their ability to infect immunocompetent individuals, these fungi are attractive targets for vaccine development. Indeed, extensive vaccine research has targeted these endemic mycoses.

Coccidioidomycosis (Valley fever) is a fungal infection caused by inhalation of a *Coccidioides* species, mainly *C. immitis* and *C. posadasii*. These dimorphic fungi grow as a mycelium in the environment (e.g., soil) and produce arthroconidia that can be dispersed and inhaled by humans when the mycelium is fractured. Inside their mammalian host and at an elevated temperature of 37°C, arthroconidia develop into spherules with endospores. Coccidioidomycosis infections are geographically restricted to the southwestern desert regions of the United States and Mexico, where they are a significant public health burden. A vaccine for coccidioidomycosis is highly desired and appears feasible, as *Coccidioides* species are primary pathogens capable of infecting healthy individuals and secondary *Coccidioides* infections are very rare, suggesting long-lasting immune protection is possible. Indeed, multiple vaccine candidates have been reported in animal models. In the 1960s formalin-killed spherules or UV-irradiated live attenuated strains were used for *Coccidioides* vaccine development. Mice immunized with formalin-killed spherules were protected against challenge with inhaled *Coccidioides* (90). This is the only vaccine for this fungus that has been evaluated in human trials. Although it was deemed safe in humans, development stopped after a phase 3 trial did not demonstrate a clear reduction of incidence and disease severity in the vaccinated group (90). Live attenuated strains derived from either UV irradiation or a *CPS1* gene deletion have also been tested and shown positive results in animal models (84). Nicely summarized in recent reviews (12, 59), live attenuated or single-antigen-component-based vaccine candidates have been tested in animal models, with encouraging results. Among these, antigen 2 has emerged as an attractive vaccine candidate. This proline-rich antigen is located under the surface of the spherule and is an effective vaccine against *Coccidioides* challenge in a murine inhalation model (56, 154). However, as *Coccidioides* cells contain multiple nuclei, making it a difficult system to study gene function, better understanding of the fungal biology and immunology will likely help in identifying a candidate and developing it into a suitable vaccine.

Blastomyces is an endemic fungus that causes blastomycosis, and the adhesion protein Bad1 is important for host-pathogen interactions. Mice had strong immune activation and increased survival when immunized with either a live attenuated *bad1*Δ mutant strain or a recombinant-Bad1-expressing yeast (144, 146). Histoplasmosis is another fungal infection, caused by the endemic fungus *Histoplasma capsulatum*. Histoplasmosis and blastomycosis are endemic in overlapping regions, with cases mostly concentrated along the Mississippi River valley in the east and Midwest of the United States. Early studies utilized cell wall extracts and fractions to confer protective immunity against otherwise lethal *H. capsulatum* challenge. Notably, a glycoprotein encoded by the heat shock gene *HSP60* was identified in the 62-kDa fraction of the cell extract. Immunization with a recombinant Hsp60 (rHsp60) protein conferred strong protection against *H. capsulatum* challenge in a murine intranasal infection model. Immunized mice displayed priming of $CD4^+$ Th1 effector responses against histoplasmosis (39). H antigen (27) and other antigens, as well as antibody-based passive immunization, have been tested for protection against histoplasmosis. The results are summarized in a recent review (107).

In conclusion, fungal vaccine development is the last frontier in vaccine development against infectious diseases and has gained significant interest in the past decade. A variety of fungal vaccine candidates, including both live attenuated and heat-killed whole-cell-based vaccines as well as

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recombinant antigen or subunit-based vaccines, have been reported for multiple fungal pathogens causing invasive mycoses (**Table 1**). However, very few of these vaccines have been evaluated in human clinical trials. It is hoped that some of these efforts will lead to an effective fungal vaccine for human clinical use in the near future.

FUTURE PERSPECTIVES

In addition to the previously described fungal vaccine candidates and platforms, several new platforms and technologies may help accelerate fungal vaccine research. The glucan-particle-based fungal vaccine delivery strategy has shown great potential (115, 116). Glucan particles are porous cell wall shells derived from the budding yeast *S. cerevisiae*. They contain primary β-1,3-glucan, which is recognized by host Dectin-1 receptors. One advantage of glucan particles is that they can be loaded with antigens, enabling delivery to phagocytes and eliciting robust and long-lasting antibody and Th1- and Th17-based protective T cell responses. Mice vaccinated with glucan particles carrying either crude antigen extracts or purified antigen proteins all showed strong protection against wild-type *C. neoformans* challenge (115). This glucan particle platform may also provide a screening opportunity to identify antigen candidates against different fungal infections.

Fungal extracellular vesicles (EVs) have been proposed as fungal vaccine candidates. EVs are lipid bilayer particles that are secreted by almost all living fungi (26, 103). In fungal pathogens like *C. neoformans*, EVs carry many immunogenic proteins, including mannoprotein MP88, chitin deacetylase Cda family proteins, and Vep proteins—all of which have been tested as vaccine candidates (101). Therefore, EVs may offer a novel approach. In a recent study, mice immunized with EVs isolated from *Cryptococcus* acapsular *cap59*Δ mutants produced antibodies against EV proteins and showed strong protection against wild-type H99 challenge (101). EVs isolated from *Candida* and *Paracoccidioides* also show positive immunogenicity and vaccine protection in animal models (73, 102). The potential advantage of an EV-based vaccine is that it is a natural mix of multiple antigens that provides strong immunity. EVs offer more stable conformational conditions for protein components that circulate in body fluids and show efficient association with antigen-presenting cells (55). However, there are potential obstacles for developing fungal-EV-based vaccines. EVs are a mixture of many components, some of which could be toxic or immunosuppressive. Additionally, given their heterogenicity, it may be challenging to produce EVs at a large scale. With continued refinement, EVs may provide a novel strategy for fungal vaccine development.

Nanoparticle technology has been proposed to enhance fungal vaccine efficacy (60). Nanoparticles can be made from different materials, including fungal cell surface carbohydrate components, lipid-based particles, and metallic nanoparticles, and are effective fungal antigen carriers. Since mRNA was discovered to induce humoral immunity in 1995, mRNA vaccine technology has been proposed to develop a new generation of vaccines against cancer and infectious diseases (14). The recent approval of two COVID-19 mRNA vaccines has accelerated the use of this technology, offering hope for more mRNA-based vaccines in the future. Both COVID-19 mRNA vaccines use a lipid nanoparticle carrier to deliver SARS-CoV-2 spike protein mRNA. Similarly, lipid nanoparticles may be utilized to deliver mRNAs of fungal cell surface proteins.

The ultimate goal for fungal vaccine development may be a pan-fungal vaccine that can protect hosts against multiple fungal infections. Several strategies have been proposed for pan-fungal vaccine development: utilization of the β-glucan from *S. cerevisiae*, identification of monoclonal antibodies (IgG2, IgM) against conserved fungal antigens, etc. Although the goal remains challenging, the combination of improved technology platforms, a better understanding of fungal immunology (e.g., trained immunity) and disease mechanisms, and increased awareness of fungal infection may soon lead to new fungal vaccine breakthroughs and adoption of these vaccines into clinical care.

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