

Vaccines in the treatment of invasive candidiasis

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Candida albicans is the most common cause of hematogenously disseminated candidiasis, and this disease is particularly prevalent in immunocompromised patients. The mortality of invasive candidiasis remains 40% to 50% even with the proper treatment with current antifungal drugs. Recently, with the better understanding of host-fungus interactions, notable progress has been made in antifungal vaccine research. Most antifungal vaccines exert protection by inducing either (or both) B-cell and T-cell responses. Here we summarize the current available information on *C. albicans* vaccines, highlight the obstacles that researchers identified, and offer several suggestions.

Introduction

Invasive candidiasis is the third to fourth most common bloodstream infection in the United States. The mortality of invasive candidiasis is 40% to 50%, even with proper antifungal therapies.¹ In China, the incidence (from 4.12% to 41.18%) and mortality (from 9.8% to 60.0%) of opportunistic invasive fungal infections are somewhat different.² Overall *Candida albicans* is the most common *Candida* species causing candidemia, followed by *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei*. *C. albicans* is fundamentally a human commensal and its interaction with the host immune system plays an important role in both commensalism and infection. Systemic candidiasis occurs predominantly as a consequence of some high-risk medical procedures like central venous catheters, immunosuppressive therapy, surgery, and exposure to broad spectrum antibiotics.^{3–9}

In recent years, a number of publications have confirmed the immunogenicity and efficacy of vaccines against candidiasis in animal models,^{10–13} and even have tested the efficacy and safety in clinical trials.^{14,15} Fungal cell-wall polysaccharide, proteins,

and live attenuated fungi have been investigated as vaccine targets. Even considering the capital and technical barriers, bringing protective vaccines to the clinic appears promising. Here we summarize the latest candidate *Candida* vaccines and their protective mechanisms. We also offer possible approaches to strengthen vaccines' triggering of the host immune response.

Live Attenuated *C. albicans* Strain

Saville et al.^{16,17} selected a genetically engineered *C. albicans* *tet-NRG1* strain as an attenuated vaccine against disseminated candidiasis. In general, the morphogenetic transition between yeast and filamentous growth is one of the important virulence factors in *C. albicans*. *NRG1* is a negative regulator of filamentation in *C. albicans*.¹⁶ In the *tet-NRG1* strain, the expression level of *NRG1* can be regulated by doxycycline (DOX) both *in vitro* and *in vivo*. Without the antibiotic, the high level of *NRG1* blocks the yeast-to-hyphal transition, while DOX treatment inhibits the expression of *NRG1* to allow hyphal formation. In the model, the *C. albicans* *tet-NRG1* strain was grown overnight without DOX, then 1.7×10^6 cells in 200 μ l saline were injected into female mice via the lateral tail vein. Two weeks later, all mice received 5.2×10^5 cells of a fully virulent *C. albicans* CAF-2 strain. The survival fraction was monitored during the following 16 d. The mice immunized with the *tet-NRG1* strain were 100% viable compared to 100% mortality in the saline group. Furthermore, B-cell-deficient, T-cell-deficient, and neutropenic (DBA/2N) mice were used to evaluate the efficiency of the *tet-NRG1* strain with the same vaccination approach. Both the B-cell-deficient and DBA/2N mice showed 100% survival versus 100% mortality in the control mice, while the vaccine failed to exert protection in the T-cell-deficient mice. According to these experiments, the protection of the attenuated *tet-NRG1* strain relied on a T-cell-mediated adaptive immune response.¹⁷ In our laboratory, an attenuated *C. albicans* strain (CaLY55) has shown an exciting protective effect against disseminated candidiasis in mice (unpublished data). However, because of the complexity of their antigens, applying attenuated vaccines in the clinic will challenge the safety requirements of the FDA.

Protein Vaccines

Live attenuated *C. albicans* strains have complex antigens that have not been well characterized, leading to restrictions in their

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application as vaccines. Thoroughly studied protein vaccine candidates such as Sap2p and Als3p are simple and have clearly defined amino acid sequences. Protein vaccines are potentially safer than the attenuated *C. albicans* strain and have a wider scope of application in patients.^{14,18,19}

Sap2p

The secreted aspartyl proteinase (SAP) family has at least 10 different members, designated Sap1p to Sap10p.^{20,21} Naglik et al.²² analyzed gene expression in over 130 subjects with oral and vaginal *C. albicans* infections and found that *SAP2* and *SAP5* were the most common genes expressed. Other research²³ reached a similar conclusion - that *SAP2*, *SAP4-6* and *SAP7* were the predominant proteinases expressed in patients both with vulvovaginal candidiasis (VVC) and recurrent vulvovaginal candidiasis (RVVC). In the candidal vaginitis model,²⁴ rats were immunized with 100 µg SAP, in which Sap2p is the majority protein, via intravaginal (i.vag.) or intranasal (i.n.) routes, with or without cholera toxin (CT). SAP vaccination resulted in significant fungal clearance in vagina, and 3 weeks after the infection no fungus was detected in the vaccination group compared to the control. Both administration routes for SAP vaccination resulted in the similar protection, and induced protective IgA antibodies when added with CT.

Recently, research reported by Bernardis et al.²⁵ demonstrated a protective Sap2 protein vaccine, PEV7. PEV7 is a truncated, recombinant *C. albicans* Sap2p consisting of amino acids 77 to 400 incorporated into the lipid bilayer of the influenza virosomes, and is designed to protect against RVVC. In the preclinical studies, IgG specific for Sap2p, was significantly induced in NMRI mice immunized with PEV7 via intramuscular (i.m.) injection compared to the control mice. In a rat model of candidal vaginitis, PEV7 generated adequate and persistent protection against *C. albicans* after i.m. immunization whereas i.vag. resulted in a lower response. Furthermore, the PEV7-immunized rats had prolonged survival times and accelerated clearance of fungi. Most of all, PEV7 was also found to be safe in a repeated-dose toxicological study in rats. According to Edwards's review article,¹⁴ a Sap2p vaccine is one of the more promising candidates and has gone through Phase I clinical trials against *C. albicans* infection. This active vaccine, which is now in development by Pevion Biotech, prevents vaginitis caused by *C. albicans* and shows both tolerability and efficacy in humans.

Als1p and Als3p

Agglutinin-like sequence (Als) proteins locate to the surface of *C. albicans* and play important roles in the pathogen's adhesion to human endothelial cells and in the development of invasive candidiasis.²⁶⁻²⁹ Edwards and his co-workers have explored several vaccines against invasive candidiasis, including rAls1p-N (recombinant N-terminus of Als1p),^{10,11,30} rAls3p-N (recombinant N-terminus of Als3p),^{31,32} and NDV-3 (recombinant N-terminus of Als3p formulated with alum adjuvant).^{13,15}

rAls1p-N (amino acids 17 to 432 of Als1p) was produced in *Saccharomyces cerevisiae* by recombinant technology and purified

by gel filtration and Ni-nitrilotriacetic acid matrix affinity purification. Mice were immunized with rAls1p-N and complete Freund's adjuvant subcutaneously (s.c.) at day 0, then boosted with different dosage of rAls1p-N with incomplete Freund's adjuvant at day 21. Two weeks after the boost, vaccinated mice and the control mice were infected with a lethal inoculum of *C. albicans*.¹⁰ In the following 30 days, rAls1p-N vaccination improved the survival rate to 50%–57% compared to 100% lethality in the controls, and decreased significantly the fungal burden in the kidney. Meanwhile, mice immunized with the same dosage of rAls1p-N intraperitoneally (i.p.) resulted in 25% survival.³⁰ These experiments remind us that vaccines should have a proper administration method. In particular, rAls1p-N vaccination still had significant protective effects in neutropenic mice, B-cell-deficient mice, and in murine models of oropharyngeal candidiasis and *Candida* vaginitis. According to these experiments, the protective mechanism of rAls1p-N vaccination relied on T cells rather than B cells.

Als3p is involved in adherence, biofilm formation, and cell invasion.^{33,34} rAls3p-N (amino acids 17 to 432 of Als3p) vaccination induced a stronger antibody response than rAls1p-N and provided a similar cell-mediated protection efficacy when combating oropharyngeal or vaginal candidiasis.³⁴ Mice were immunized with rAls3p-N in the same way as rAls1p-N. In the murine oropharyngeal candidiasis model, rAls3p-N vaccination significantly reduced the tongue fungal burden and inflammatory response. Similarly, in a model of *Candida* vaginitis, rAls3p-N vaccination generated a remarkable reduction in vaginal fungal burden. In the mouse model of disseminated candidiasis, rAls3p-N vaccination notably improved the survival time. However, since the rAls3p-N-vaccinated mice that did not survive had high antibody titers, the antibody titers did not significantly correlate with a protection effect. Compared with rAls1p-N, rAls3p-N vaccination was more effective in treating oropharyngeal candidiasis.

To further clarify the protective mechanism of rAls3p-N, the authors conducted a series of experiments to explore the mechanism of rAls3p-N vaccine plus aluminum hydroxide (Al(OH)₃) adjuvant in a mouse candidiasis model.³² IFN-γ-deficient mice immunized with CD4⁺ lymphocytes from the rAls3p-N-vaccinated wild type donor mice had approximately 40% survival, indicating that CD4⁺ lymphocyte-derived IFN-γ was important in combatting *C. albicans*. In cyclophosphamide-induced neutropenic or gp^{91phox-/-} deficient mice (unable to generate superoxide and have impaired function in killing microbes), no protection was created by rAls3p-N vaccination, suggesting that neutrophils play an essential role in the protection. Moreover, in IL-17A-deficient mice, there was no vaccine-induced protection either when compared to the control mice. Then the vaccinated IL-17A-deficient mice and the vaccinated wild-type mice were cross-adoptively transferred CD4⁺ cells. As expected, the transfer of CD4⁺ cells from the wild type mice conferred higher protection than transfers from the IL-17A-deficient mice. According to these experiments, IL-17A derived from CD4⁺ lymphocytes was necessary for protection. In addition, the protective mechanism also involved many cytokines; for example,

the neutrophil activating chemokines KC and MIP-1a. In the rAls3p-N vaccinated mice, higher levels of Th1, Th17 and Th1/17 cells were detected than in the control mice.

Taken together, the rAls3p-N vaccine improved protection against *C. albicans* infection in mice by inducing Th1, Th17, and Th1/17 lymphocytes, thereby produced cytokines and the recruitment of neutrophils to the infection sites. IL-17A was not necessary for defense against *C. albicans* challenge in non-vaccine immune defense, but was necessary in vaccine-induced protection.

rAls3p-N formulated with alum adjuvant (NDV-3) has been tested in a Phase I clinical trial, and has shown to be well tolerated even at 300 mg.¹⁵ The majority of the subjects had a robust humoral immune response, and elicited high titres of antibody within 3–7 d of administration. As well, the production of IFN- γ and IL-17A was increased. These observations suggest that in humans, NDV-3 stimulated T cells, B cells and antibody responses.

Hsp90p

Heat shock protein 90 (Hsp90), a stress-induced cellular chaperone, is highly conserved within *Candida* species.^{35,36} An Hsp90 DNA vaccine (1 μ g) was tested with 2 administration methods, i.m. and intradermal (i.d.) injection.³⁷ Mice vaccinated with a DNA vaccine via i.d. resulted in 64% prolonged survival times compared to a PBS control, whereas vaccination via i.m. did not lead to protection. As well, recombinant protein r-hsp90-CA resulted in a 118% increase in survival compared with the PBS control group. Moreover, Mycograb[®] (Neu Tec Pharma) is a monoclonal antibody binds to the immunodominant epitope of *C. albicans* Hsp90p. Mycograb consists of the variable ends of the heavy and light chains which were linked together by 2 cross-cysteine bonds.³⁸ In preclinical studies, results from checkerboard methods demonstrated the synergistic action of Mycograb with amphotericin B (AMB) against both fluconazole-sensitive and fluconazole-resistant *C. albicans* strains. In a mouse model of systemic candidiasis, 2 mg/kg of Mycograb in combination with AMB improved the killing of *Candida* compared with AMB treatment alone.³⁹ In a multicenter double blind placebo-controlled trial of patients with invasive candidiasis, complete mycological resolution reached 84% in the combination therapy group compared to 48% in the AMB monotherapy group. Moreover, the clearance of infections in the combination therapy group was twice that for the AMB therapy alone, resulting in mortality of 18% compared to 4%. Although the Committee for Medicinal Products for Human Use (CHMP) adopted a negative opinion because of both quality and safety concerns, monoclonal antibodies in the treatment of invasive candidiasis is still a promising approach.³⁸

Hyr1p

Hyr1p is a glycosyl phosphatidylinositol (GPI)-anchor mannanoprotein on the cell wall of *C. albicans*^{40,41} and enhances *C. albicans* resistance to neutrophil killing.⁴² rHyr1p-N (amino acids 25–350 of Hyr1p) was produced in an *Escherichia coli* pQE-32 expression system, and purified by a 6 \times His tag.⁴²

Mice were immunized with 20 μ g rHyr1p-N in complete Freund's adjuvant via subcutaneous injection and boosted on day 21 with incomplete Freund's adjuvant. Fourteen days after the boost, mice were infected with 2.2×10^5 cells of *C. albicans* SC5314. Meanwhile, alum was also evaluated as an adjuvant in vaccine protection. Mice were immunized with 33 μ g rHyr1p-N in 0.1% alhydrogel and then infected with 7×10^5 cells of *C. albicans* strain 15563. The results showed that vaccination with rHyr1p-N mixed with adjuvant markedly improved survival to 60% or 70%. However, so far there has been no specific information available about its behavior in preclinical studies.

Cell wall extract

In Thomas's study,⁴³ mice were vaccinated subcutaneously with cell wall proteins obtained through β -mercaptoethanol (β -ME) extraction of *C. albicans*. In the immunization group, the β -ME extract (40 μ g) was injected subcutaneously combined with Ribi Adjuvant System (RAS) R-700. After 3 weeks, mice in the vaccinated group received a booster injection in the same way. Eight days after the booster injection, each mouse received 1×10^6 *C. albicans* cells via intravenous injection. Some mice were sacrificed on day 3 after injection, and kidneys and blood were collected to further evaluate the vaccine. The remaining mice were monitored for survival. 75% of the vaccinated mice survived during the 28 day observation period while the unvaccinated mice all died. The results of colony formation units (CFUs) and histopathological analysis (GMS staining) in the kidneys showed that the *C. albicans* β -ME extract significantly decreased the tissue fungal burden and inflammatory lesions when compared to the control mice. According to the ELISA test of the anti-sera, vaccination elicited both high IgG and IgM responses. Two-DE and mass spectra were further used to identify a total of 20 unique antigenic proteins in β -ME extract, among which Mp58/Pra1p is the most abundant protein. As well, the β -ME extract also included glycolytic enzymes and heat shock proteins. In such cases, proteomic techniques can identify the diversity of proteins present in vaccines derived from cell wall extracts.

Glycoconjugates Vaccines

The combination of polysaccharide with protein, in which the polysaccharide acts as a carrier, could elicit T cell response and have the potential to directly inhibit fungal growth. This approach can broaden the spectrum of protection and decrease the chances of immune evasion.^{18,44–50}

β -Mannan and peptide conjugates

An innovative β -mannan vaccine has been generated by Xin et al.⁵¹ The glycopeptide vaccines were created by combining β -mannan and peptide epitopes. The linker was a non-immunogenic, cross-coupling reagent, which resulted in a stable amide bond. Six T-cell peptides found in *C. albicans* cell wall proteins were selected by algorithm peptide epitope searches; each was synthesized and conjugated to the fungal cell wall

β -mannan trisaccharide [β -(Man)₃] by a novel saccharide-peptide linker chemistry to create glycopeptide conjugates. The vaccination of β -(Man)₃-Fba, β -(Man)₃-Met6 or β -(Man)₃-Hwp1 each induced significant protection against experimental disseminated candidiasis in mice, and reduced kidney fungal burden, while the vaccination of the β -(Man)₃-Eno1 or β -(Man)₃-Gap1 gave moderate protection, and the β -(Man)₃-Pgl1 conjugate slightly enhanced candidiasis. The immunized groups that received the β -(Man)₃-Hwp1, β -(Man)₃-Fba, or β -(Man)₃-Met6 conjugates showed 80–100% survival throughout the 120 day post-challenge observation period. This original approach employing fully chemically synthetic and defined immunogens has widespread implications for the development of vaccines. Recently, Xin et al.⁵² have shown that a peptide alone (Fba) is highly protective as an active vaccine against several *C. albicans* strains.

β -Glucan conjugate vaccine

β -glucan conjugated with the MF59 adjuvant, which has been used extensively in influenza vaccines in Europe, but is not approved by the FDA for use in the United States, showed excellent protection in the murine vaginal candidiasis model.⁵³ This protection was due to the anti- β -glucan IgG in serum and in the vagina. Another glucan vaccine is β -glucan-CRM197 which is a laminarin-diphtheria toxoid (CRM197) conjugate vaccine Lam-CRM197.⁵⁴ When Lam-CRM197 was administered with MF59, the mortality of the infected mice decreased. Passive transfer experiments demonstrated that the protection in an invasive candidiasis model derived through such vaccination was mediated by antibodies.

Attempted Approaches to Strengthen Vaccines' Immune Response Stimulation

As mentioned above, we know that current *C. albicans* vaccines play important roles in the prevention of candidiasis, which are primarily due to their reinforcement of immunogenicity. Here, we give some suggestions for the production of the ideal vaccine that can elicit strong immune response.

Target selection

Fungal cell walls are indispensable organelles and are composed of molecules that are largely absent in mammals. The cell wall plays an important role in continuing cell wall biosynthesis, in growth, in budding in *C. albicans* and especially in crucial host-fungus interactions that facilitate the establishment of human mycoses. Most virulence factors, including those involved in adhesion, invasion, and the yeast-to-hypha transition, localize at the cell wall, and include proteins such as Hsp90p, Sap2p, Als1p and Als3p. Thus cell wall proteins might be ideal vaccine targets for inducing a protective immune response in the host.

Glycosylation of the vaccine

Since recombinant proteins have been generally produced in *E. coli*, the expressed proteins aren't glycosylated as they would

be in eukaryotic cells, with the result that they differ from the natural structure. Although non-glycosylated proteins are able to mediate protective immunity, glycoconjugates (mannans and β -glucans) have been tested to establish whether it is necessary to be glycosylated to enhance immune response. Specht et al.⁵⁵ have established the role of mannosylation in inducing protection with protein vaccines against *Cryptococcus* by comparing the protective efficacy of the mannosylated and unmannosylated recombinant proteins produced separately by *Pichia pastoris* and *E. coli*. Both recombinant proteins generate immune protection against *Cryptococcus* infection. However, the mannosylated protein has the additional ability to induce a potent T cell-mediated immune response, and to protect against infection. Moreover, yeast-mannosylated ovalbumin induced greater T cell proliferation than unmannosylated ovalbumin produced in *E. coli*; this effect was attributed to O-mannosylation rather than N-mannosylation. Mannosylation enhanced both CD8⁺ T cell proliferation, and the secretion of proinflammatory cytokines, such as tumor necrosis factor (TNF) and IL-12. However, in the case of chemical deglycosylation, the mannosylated protein would turn into unmannosylated protein, leading to the disappearance of the above effects of mannosylation.^{47,50} Hence, most attention should be directed at the study of the vaccines with polysaccharides or glycoconjugates against *C. albicans*, which are based on β -mannan, β -glucan and their oligosaccharide synthesis.

Suitable adjuvants

The majority of immune response enhancement methods are based on the vaccine itself in the design and production stages. However, the adjuvant is also a key factor in enhancing the immune response by promoting the antigenicity of an immunogen. It causes substantial and additional activation of T-cells or B-cells. In recent years, Freund's adjuvant has been extensively used in animals, especially in mice models. But now it's no longer recommended because of its painful reaction and potential tissue damage. Instead, the alum adjuvant is widely used in humans. In the future, in order to achieve better efficacy without side effects, new adjuvants or modified adjuvants should be tested.

Delivery by dendritic cells

Dendritic cells (DCs) are antigen-presenting cells that have a branching or dendritic morphology and have phagocytic functions; DCs connect innate immunity with adaptive immunity. Using DCs as vehicles to present vaccines to T cells would promote the proliferation of specific antigens and the cell-mediated immune response.⁵ The localization of DC vaccination is different depending on the injection process. Intravenous injection leads to DC localization in the reticuloendothelial system and the lungs, whereas subcutaneous injection targets DCs to the lymphatic system. DCs delivery shows advantages in immunocompromised patients over others.⁵⁶

Apart from DCs delivery, there are some other carriers, including nasal delivery systems, lipid particle delivery systems, DNA delivery, and virosome delivery, which are all approaches to enhance the activity of *C. albicans* vaccines.¹⁴

Combine univalent vaccines into a multivalent vaccine

At present, most *C. albicans* vaccines are univalent. Since *C. albicans* possess many kinds of virulence factors, and one vaccine is usually specific to a single factor, other virulence factors would compensate, allowing *C. albicans* to escape from the immune response. In contrast, a multivalent vaccine that contains more than one unrelated antigen will provide better protection by eliciting effective immune responses and protective antibodies, as well as reducing the incidence of fungal immune evasion. Casone¹⁸ has proposed a constructive idea to combine 2 unrelated, major virulence-associated immunogens as a multivalent vaccine. The recombinant antigens Als3p and Sap2p, which are currently in clinical trials, would be the first combination to explore. Although there are several technical and financial difficulties, production of multivalent vaccines is still a promising way to overcome *C. albicans* infection.

Passive immunotherapy

A significant challenge to the development of effective vaccines will be to elicit immune responses in immunocompromised individuals who are most at risk for invasive fungal infections. In these vulnerable patients, the vaccine usually elicits a weak immune response or nothing, or even generates side effects. In this circumstance, passive immunotherapy could be considered, which would involve opsonisation, complement fixation, immune response and protective antibodies.⁵ At present, monoclonal antibody C7 (MAb C7)^{43,57,58} and Mycograb³⁹ are both successful cases. mAb C7 was produced by standard methods with splenocytes from BALB/c mice immunized with intraperitoneal injections of a *C. albicans* high-molecular-weight stress mannoside protein recognized by salivary secretory immunoglobulin A (sIgA). MAb C7 exhibited a potent antifungal effect through inhibiting both germination and the adhesion of *C. albicans* to Hep-2 monolayers and BECs, and through direct candidacidal activity. Further studies claim that mAb C7 binds to the N terminus of Als3p and functions through the blockage of the reductive iron uptake pathway in *C. albicans*. Mycograb, as mentioned in section 3.3, binds to *C. albicans* Hsp90p and shows synergistic fungicidal effect with AMB in patients with invasive candidiasis. Antibodies will offer good prospects in immunotherapy especially in immunocompromised individuals. Full assessment of the efficacy in prevention, treatment, prognosis and recurrence would be helpful to determine the best drug administration period.

Problems and Proposals

Many positive results have been achieved in the current development of vaccines. Nevertheless, there are still several problems for us to solve - the following are the crucial points: (1) The vaccines show marked protection in animal models, but could be less effective in human; (2) The protection of the vaccine is not persistent or high-grade; (3) The vaccine could cause unacceptable toxicity, which brings new troubles in treatment; (4) There is no perfect standardized manufacturing process; (5) The clinical

trials need many volunteers, involve high costs and have a long cycle; (6) Vaccines have a common disadvantage with biologics, it is difficult to maintain stability during the production, transportation, and storage processes.

Further understanding of the mechanisms of the *C. albicans*-host interaction and identification of some new targets would be of considerable importance. Then, in the preclinical trials, researchers need to consider:⁹ (1) Using different species of animals, including mice, rabbits and monkeys, to test the effectiveness of vaccines; (2) Because hosts have different physical status, we should simulate many animal models to evaluate the efficacy of vaccines. These infection models could include immune-normal animals, immune-deficient animals, and could involve variations in the symbiotic bacteria in cross-infection animals; (3) To ensure the susceptibility of *C. albicans* before experiment; (4) The dose of *C. albicans* should be controlled at the required smallest inocula to infect the majority of animals and produce the intended outcome of death or tissue infection; (5) Observe the dosage and delivery time of the vaccines to produce the greatest protection effect; (6) Distinguish various routes of *Candida* infection; (7) Test multiple parameters, such as tissue CFU and survival rates.

When the vaccines come to clinical trials, researchers should determine the appropriate dosing regimen in real-time tracking feedback according to the tolerance, pharmacokinetics, therapeutic effect and safety. Meanwhile, both the efficacy of the vaccine alone and the synergy of the other antifungal drugs should also be examined.

In summary, the traditional antifungal chemotherapy against systemic *C. albicans* infections has met some limitations, such as the toxicity and emergence of resistance to the limited options of antifungal drugs. Vaccines can represent novel approaches against *C. albicans* infection. Although the currently available vaccines have demonstrated good protection, there still exist problems such as how to elicit immune responses in immunocompromised individuals. Thus there is still long way to go to finally achieve a vaccine applied in patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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