Review Article

Vaccines for fungal infections

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ABSTRACT

High morbidity and mortality caused by mycotic infections has been a cause for concern. Trials for various vaccines against fungal pathogens have not been approved by the US Food and Drugs Administration because of the high cost of production and lack of a single suitable candidate. Most fungal infections require cell-mediated immunity for their clearance. This has been the basis for the development of various vaccines. We discuss the various trials of candidate vaccines, the protective efficacy as well as their shortcomings. Recent research suggests that a universal vaccine can be prepared which may be effective against most fungal pathogens.

Natl Med J India 2015;28:14–19

INTRODUCTION

The incidence of invasive fungal infections has increased during the past decade. The proportion of vulnerable patients is increasing, paralleling the excess use of immunosuppressive therapies. The conditions that predispose patients to fungal infection include haematopoietic stem cell transplantation, solid organ transplantation, chemotherapy, preterm birth, etc. Treatment approaches for common fungal infections such as Candida and Cryptococcus have been well studied, but the number of clinical trials and management strategies for other invasive fungal agents have been few. Vaccine development is a key for progress in the management of fungal infections.

The morbidity due to mycotic infections is high, especially due to invasive fungal infections. Also, high mortality rates have been reported in many infections despite antifungal therapy.[1] Increasing resistance to antifungal agents and increasing healthcare costs to treat fungal diseases are additional issues. The emergence of immunodeficiency states such as HIV further compounds the situation. Therefore, preventing fungal infections has become important.

FUNGAL VACCINES

The primary objective of most vaccine trials is to enhance the ability of the immune system to clear the tissues of all fungal elements. Natural immune responses are capable of clearing the infection in most cases but a small focus of infection usually remains in the tissues, especially in cases of invasive fungal infections. Therefore, efforts also need to be made to prevent the establishment of the dormant state in the host. Alternatively, a vaccine that prevents reactivation of already dormant fungi may be more useful in already infected individuals.[2]

Despite extensive investigations of these vaccines, none has yet been approved by the US Food and Drugs Administration (FDA) for use in humans.[3] There are many hindrances to the production of an effective vaccine against fungal infections. There is an under-appreciation of various fungal diseases. This is because the majority of infections are asymptomatic, manifesting only when mucosal defenses are breached or when the immune responses fail to clear the pathogen. However, even in symptomatic patients, serious sequelae are rare. The high cost of antigen production and the high cost of conducting phase 1 clinical trials for establishing tolerability and safety is another problem. The lowered immune status of the patient, especially in invasive fungal diseases, further adds to the complexity of the situation.

FUNGAL IMMUNITY

Innate immunity

Innate immunity is defined as the component of the immune system that utilizes germ-line encoded molecules to eliminate all foreign substances. It lacks memory and is composed of physical barriers, soluble mediators (e.g. complement) and various cell populations including macrophages, neutrophils, dendritic cells and natural killer (NK) cells. Neutrophils are essential for host defence against *Candida, Fusarium* and *Aspergillus*. They have phagocytic and microbicidal action along with secretion of cytokines.[4] Macrophages are phagocytic cells that have fungistatic as well as fungicidal functions, produce cytokines and chemokines and present antigens to both CD8+ and CD4+ cells. Some intracellular fungi such as *Cyrptococcus neoformans, Histoplasma capsulatum* and *Blastomyces dermatitidis* thrive within the macrophages, a niche that protects them from host defences.[5] Monocytes and macrophages mainly produce interleukin (IL)-1, IL-10, IL-12 and tumour necrosis factor (TNF)-α while NK cells are capable of producing IL-12, TNF-α and interferon (IFN)-γ.[6] Dendritic cells engulf and kill fungal species by mobilization of phagolysosomes, presentation of fungal antigens and activation of various signaling pathways.[7] The non-cellular effectors of innate immunity include collectins, complement and antibodies. These molecules mediate opsonization thereby promoting ingestion of the fungus by phagocytes.[8]

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Acquired immunity

Humoral immunity requires recognition of specific foreign molecules that possess memory or recall for the molecules which initially activated them. It is best seen with *Candida albicans* and *Cryptococcus neoformans*.[9] The antibodies produced in response to an antigen act as opsonins that enhance the phagocytosis of fungi. They promote agglutination of fungal elements and antibodies to fungal surfaces block binding to host cells, which may limit the extent of infection. Apart from all these functions, they also exert a direct fungicidal effect.[10]

Cell-mediated immunity, on the other hand, plays an important role in the pathogenesis of invasive fungal infections, viz. *B. dermatitidis, C. immitis, C. neoformans, C. albicans, H. capsulatum, Paracoccidioides brasiliensis* and *Pneumocystis jirovecii*.[2] The principal mechanism by which CD4+ T cells influence host resistance is by production of cytokines. Naïve CD4 cells in the presence of various microenvironmental stimuli differentiate into various subsets. T-helper 1 (Th1) cells produce IFN-γ, IL-2, TNF-α and IL-12 which are responsible for clearance of infection, especially in primary disease.[11] Th2 cells release IL-4, IL-5, IL-10 and IL-13. These cytokines block the Th1 response, thereby balancing the immune pathology. Increase in Th2 cytokines is observed in progressive disease and neutralization of their activity restores protective immunity.[12] Th17 cells produce IL-17, IL-21 and IL-22.[13] IL-17A has been found to have a pro-inflammatory role which helps in controlling infections with *C. albicans* and *P. jirovecii.* Cytotoxic activity of the CD8+ T cells is responsible for damaging the hyphae of *C. albicans* and *C. neoformans* cells directly.[14] T cells are also involved in the class-switching of antibodies. Th1 cells promote the synthesis of IgG2a and IgG3, whereas Th2 cells enhance the production of IgM, IgG1, IgA and IgE.[15]

VACCINATION STRATEGIES

Conventional methods

Live attenuated vaccines have been used against various fungi such as *Candida, Coccidioides* and *Blastomyces dermatitidis.* Inert fungal substances such as proteins and carbohydrates have been used for vaccination. The use of such components is safe as they do not involve the use of replicating microbes whose virulence may be altered during the course of infection on the vaccinee. However, the yield of the native antigen is low and requires purification. Therefore, the use of recombinant antigen is preferred. The immunogenic determinants of the antigen that can elicit an immune response are utilized thereby eliminating irrelevant moieties of the protein, e.g. heat shock protein 60 from *H. capsulatum* and p55 from *P. jirovecii*.

Newer methods

DNA vaccination at present utilizes a plasmid-encoded DNA that does not replicate and is injected into a muscle or bombarded into the skin. DNA delivery via gene-gun primarily induces a Th2 response whereas DNA delivered by intramuscular injection produces a Th1 response. One of the attractive features of genetic vaccination is that the genes are often expressed within the endogenous pathway of antigen processing, which will lead to cytolytic T-cell activation. Thus, genetic vaccination is superior to the use of conventional proteins which will enter the exogenous pathway, thus leading to activation of CD4 T cells almost exclusively. In several systems, DNA vaccination has induced both cellular and humoral immune responses and has generated a protective immune response.[16] This approach has several advantages it does not require prior knowledge of the antigen and it eliminates the risk of a live vaccine. The use of a larger genome, an inevitable accomplice with pathogenic fungi, might not be as fruitful as with pathogens that possess smaller genomes. The more complex the genome, the more likely it is to discover antigens that suppress immune responses rather than promote them. Another method that can be used is transfection of dendritic cells, which are potent antigen-presenting cells, with naked DNA and assess the impact on protective immunity.[17] This approach is now used for pathogenic fungi.

CANDIDATE VACCINES

Candida albicans

Candida albicans has become a major cause of morbidity among patients infected with HIV, patients in intensive care units (ICUs) or those who have undergone chemotherapy for malignant diseases or receiving immunosuppresants after transplantation. Candidaemia is now the fourth most common bloodstream infection in hospitalized patients in the USA and Europe. It has a high mortality of up to 40%, despite antifungal therapy.[1] However, vaccination is promising as the various risk groups are clearly defined.

Many vaccines for *Candida* have been described, and two have entered phase 1 clinical trials. The first vaccine utilizes a cell surface, GP1 protein member of *Agglutinin-like sequence (Als)* adhesion family. Vaccination with the recombinant N-termini of the candidal surface adhesins Als1p or Als3p (rAls1p-N or Als3p-N) protected mice from disseminated candidiasis, and reduced fungal burden in the vaginitis model and in the steroid-treated oropharyngeal candidiasis model.[18] Effective vaccination required an intact proinflammatory type 1 immune response, i.e. Th1 and Th17 responses. Th17 cells mediate phagocyte recruitment and activation of phagocytes at sites of infection. Vaccine efficacy did not require Th2 responses and was independent of the antibody or B cells.[19] These studies were advanced into a trial in non-human primates to determine the extent of IgG response when rAls1p-N was given with aluminium hydroxide as the adjuvant. IgG titres rose by 1–2 logs in these primates, which, unlike mice, are not naive to *Candida*. A phase 1 clinical trial in human subjects included 30 subjects who were given two doses of vaccine, 30 μg and 300 μg. The vaccine did not cause any adverse events and was well tolerated. The results showed a robust production of IgG and IgA1 antibodies along with an increase in production of IFN-ã as well as IL-17A. These observations suggest that in humans, both humoral and cell-mediated immunity were stimulated by the vaccine.[3]

Another active vaccine that has been through a phase 1 trial and had shown tolerability and efficacy in humans, is Sap2p that prevents vaginitis caused by *C. albicans*. It is a virosomal formulation of a recombinant secretory aspartyl proteinase (Sap2) of *C. albicans*. It was delivered by both intramuscular and intravaginal forms with a proprietary virosome. This active vaccine is being developed by a Switzerland-based company, and interim data indicate that it is well tolerated and effective at low doses.[3]

There are other vaccines currently in preclinical trials in various animal models. rHyr1p-N is a prophylactic recombinant vaccine against the N-terminal region of Hyr1p. *HYR1*, a member of the *IFF* gene family of *C. albicans*, encodes a cell surface glycosyl phosphatidyl inositol (GPI)-anchored protein that is expressed during hyphal formation and is found to mediate resistance to phagocyte killing *in vitro*. Immunization with rHyr1p-N had shown efficacy in animal models at doses 10–30 times less than those used

for rAls3p-N resulting in the production of neutralizing antibodies. It was found to be efficacious in a disseminated candidiasis and neutropenic mouse model. In a mouse model of disseminated candidiasis, vaccination with rHyr1p-N with an Alhydrogel adjuvant resulted in increased survival. These findings suggest that Hyr1p is a promising target for both active and passive immunization. The vaccine is efficacious against various strains of *C. albicans* as well as non-*C. albicans* species.[20]

A genetically engineered non-pathogenic *Candida albicans* tet-NRG1 strain had been used as an experimental live attenuated vaccine against haematogenously disseminated candidiasis.[21] NRG1 is a negative regulator of filamentation of *Candida*. In this engineered strain the gene is under control of a tetracycline regulatable promoter. Mice were first infected with this strain as a live attenuated vaccine. A secondary infection with the strain, with the virulence of the organism restored by feeding the mice doxycycline in their drinking water (downregulating NRG1, and allowing germination to occur), resulted in substantial protection from the virulence restored strain. The results indicated the highest level of protection for an anti-*C. albicans* vaccine, especially in immunocompromised mice.

A protein conjugate vaccine consisting of *laminaran (algal glucan)* linked to diphtheria toxoid as a carrier protein resulted in significant protection against disseminated candidiasis and vaginal candidiasis in murine models. The vaccine was cross protective against *Aspergillus* as well, suggesting it may be effective against all fungi that contain glucan in their cell walls.[22] Surface mannans function as adhesins and have been shown to induce a protective immune response in a murine model of disseminated candidiasis via the exaggeration of humoral immunity.[23] *Mannoprotein fraction (MP-F2)* is a cell wall fraction which preferentially stimulates CD4+ T cells to produce IFN-γ but not IL-4. Immunization of mice with MP-F2 conferred a modest reduction in candidal colony forming units (CFUs) and prolongation of mean survival time compared to controls. Other antigens include candidal heat shock proteins, other candidal surface proteins such as HYR1 and enolase.[24,25]

Aspergillus spp.

Aspergillus is the second most common cause of nosocomial, invasive fungal infections after *Candida*, with a higher mortality rate of up to 80%, despite antifungal therapy.[26] The risk factors for aspergillosis include severe depletion of white blood cell levels from cancer chemotherapy, leukaemia, bone marrow transplantation and high doses of corticosteroids or other immunosuppressants in patients receiving organ transplants or those with severe rheumatic or other autoimmune diseases. The main barrier to effective vaccination is that patients with invasive aspergillosis are immunocompromised with a lowered cellular and humoral immunity. However, various candidate vaccines are currently being tested in preclinical settings.

Vaccination in mice with freeze–thawed hyphae and culture supernatants of *Aspergillus* was tried and the allergen Asp f3 was identified as the likely immunogen. Synthetic recombinant Asp f3 was able to establish protection in mice. The protective epitopes of the truncated proteins that were responsible for T-cell Asp f3 specific stimulation were defined.[27] It was found that fungal Asp f3 is inaccessible to antibodies unless both cell walls and membranes have been permeabilized. Vaccine-primed CD4+ T cells are not expected to clear the fungal pathogen directly; however, they may activate immunosuppressed phagocytes locally that elicit the antifungal effect.

Another antigen Asp 16f is a likely candidate vaccine. Intranasal administration of recombinant antigen in conjunction with CpG oligonucleotides was shown to improve the survival of cyclophosphamide-treated mice subsequently infected with inhaled *A. fumigates.*[28] *Ex vivo* dendritic cells pulsed with *Aspergillus*, or fungal RNA, induced Th1 cell polarization and improved survival of allogenic stem cell transplanted mice infected with *A. fumigatus*.[29] Mouse dendritic cells transfected with an IL-12 expressing adenoviral vector and exposed to *A. fumigatus* stimulated type 1 immune responses *in vivo*.[30] Glucan from *Laminaria digitata* conjugated with the diphtheria toxoid CRM197 has been effective in protecting against an intravenous challenge with *Aspergillus* in mice.[22]

Cryptococcus neoformans

Cryptococcus causes life-threatening infections in patients with substantially compromised T-cell-mediated immunity resulting from HIV infection, congenital causes, or the use of immune-suppressing corticosteroids for transplantation, arthritis or other conditions.

One clinical trial in the past was based on glucuronoxylomannan (GXM) capsule, which is an important virulence factor that functions by suppressing the host inflammatory response and prevents opsonophagocytosis of the fungus. The capsular polysaccharide antigen is poorly immunogenic; conjugated to tetanus toxoid it stimulates the production of protective antibodies in mice. These antibodies provide for efficient phagocytosis, enhanced NK cell function and improvement in clearing capsular polysaccharide. A phase 1 trial had been done to determine the safety and efficacy of this vaccine in human subjects. Adverse reactions were negligible and HIV-infected subjects were also injected without serious side-effects. Thus, this vaccine is antigenic and appeared in this small study to be safe. However, further studies showed that the pleiotropic effects of GXM on host immunity, and the variable protective responses to GXM–carrier conjugates, militate against the use of intact GXM in human vaccine development.[31]

Protein-conjugated laminaran vaccine, used for candidiasis, cross-reacts with Cryptococcus-inducing specific antibodies that activated opsonophagocytosis and reduced fungal burden in healthy as well as neutropenic mice. Yeast-mannosylated ovalbumin induced significantly greater T cell proliferation. Luong *et al.* found a similar immunostimulatory effect of mannosylated antigens on CD8+ T cell function. Mannosylation significantly enhanced CD8+ T cell proliferation, and also secretion of pro-inflammatory cytokines, such as TNF-α and IL-12.[32] Proteins in the cell wall and cell membrane such as melanin and glucosylceramide are stimulatory for lymphocytes.

Histoplasma capsulatum

Histoplasmosis is common among patients with AIDS because of their suppressed immunity and in immunocompetent individuals past infection results in partial protection.

Vaccine candidates containing heat shock protein 60 (Hsp60) from *H. capsulatum* have conferred protection to mice given a pulmonary challenge. Recombinant Hsp60 was produced in a prokaryotic expression system and tested for its ability to protect BALB/c mice from a lethal intranasal challenge of *H. capsulatum* yeasts.[33] More recently, a heatshock protein Hsp70 has shown protection in mice. Cytokine release by spleen cells from mice vaccinated with Hsp60 produced substantially more IFN-γ, IL-10 and IL-12 than cells from mice immunized with *H. capsulatum*

recombinant Hsp70. The generation of IFN-γ, but not of IL-10, was dependent on T cells, in particular CD4 cells. Treatment of Hsp60-immunized mice with monoclonal antibody to IFN-γ, IL-10 or IL-12 in the inductive phase of vaccination was accompanied by increased recovery of yeast cells from lungs and spleens and 100% mortality. Subcutaneous injection of apoptotic phagocytes containing heat killed *Histoplasma capsulatum* results in CTL-mediated protection.[34]

Blastomyces dermatitidis

It is a dimorphic fungal pathogen, exists in the soil in a filamentous form and produces spores directly on the wall of the hyphae. Outbreaks are often associated with disruptions of soil that might lead to artificial elevation of spore and/or hyphal fragments in the air. These agents when inhaled infect human and animal hosts. In the host the fungus undergoes a phase transition to the pathogenic yeast form. Yeast form cells multiply in the lung and may cause disease in immunocompetent hosts, sometimes disseminating to the skin, central nervous system and bones.

One of the most important vaccine candidates for *Blastomyces dermatitidis* is Blastomyces adhesin 1 (BAD1) antigen, formerly known as WI-1. It is highly immunogenic stimulating both humoral and cell-mediated response. It serves as a ligand between phagocytes and the fungus and is recognized by CR3 and CD14 receptors. Immunocompromised mice on vaccination with purified BAD1 were protected from the otherwise lethal infection by overcoming deficits in CD4+ cell function by using memory CD8+ cells as sources for proinflammatory cytokines.[35] A strain of *B. dermatitidis* has been mutated through knocking out the gene encoding BAD1 and given live to beagles and foxhounds subcutaneously, without adjuvant. The safety and immunogenicity of this vaccine was established. Increased transcript levels of IFN-γ and TNF-α were found. The immune responses in this study were dependent on the dose of the vaccine.[36]

Coccidioides immitis

Coccidioidomycosis is asymptomatic in nearly two-thirds of immunocompetent individuals, while the rest experience a mild-to-moderate febrile illness that resembles community-acquired pneumonia. The risk factors for a severe pulmonary infection and dissemination to meningeal and extra-meningeal sites (skin, bone, joints) include black race, low income, pregnancy, advanced age, HIV infection and diabetes.

A mutant of *Coccidioides posadasii* with a double gene knockout, CTS2 and CTS3 (chitinase genes) was used as a live attenuated vaccine.[37] This attenuated mutant is not able to sporulate. Mice are fully protected against pulmonary coccidioidomycosis by this vaccine. The vaccinated mice, which were challenged with intranasal organisms, showed a mixed Th1, Th2 and Th17 type immune response. Functional Th17 cells were necessary for protection by the vaccine in this model, adding to growing evidence for the role of Th17 cells in the mechanism of protection of most fungal vaccines under evaluation.[38] Other antigens studied include spherule outer wall (SOW) antigen. A formalin-killed, whole-cell spherule vaccine was found to be exceptionally protective against lethal intranasal infections in mice. When this dose of formalin-killed spherule vaccine was used in a human field trial, vaccination failed to result in significantly fewer symptomatic cases of coccidioidal pneumonia than detected in those receiving a placebo.[39] A 33 KDa antigen isolated from the wall of mature spherules, is recognized by sera from humans who have recovered from infection and subjects who were vaccinated with killed spherules. It also stimulates cell-mediated immunity.[40] Recombinant protein chimera of Antigen 2/Proline-rich antigen (Ag2/PRA) cell wall antigen combined with coccidioides-specific antigen (CSA) has also been studied. There was lack of toxicity but the immunogenic potential was not significant. Therefore, further clinical trials were not done. Alkali-soluble water-soluble antigen (C-ASWS) showed only partial protection in mice.[41]

Pneumocystis jirovecii

Pneumocystis pneumonia is an important disease of immuno-compromised humans, particularly patients with HIV, and also patients with an immune system that is severely suppressed for other reasons; for example, following a bone marrow transplant.

The various candidate vaccines include a recombinant p55 antigen and glycoprotein A antigen. p55 antigen is a protein derived from rat pneumocystis without a defined function. It provides protection in corticosteroid-treated rats. The mechanisms whereby these antigens promote the protective immune response remain unknown, although it is likely that humoral immunity is dominant since T-cell function in steroid-treated animals is very depressed. Active immunization against *Pneumocystis jirovecii* with p55-v3 DNA vaccine in rats has shown encouraging results.[42] Major surface glycoprotein (MSG) or glycoprotein A is an immunodominant antigen acting as an adhesin. Spleen cells and CD4 T cells from rats naturally exposed to *P. jirovecii* were stimulated *in vitro* with native MSG and transferred to corticosteroid-treated rats with pneumocystosis. The organism burden was significantly reduced compared to that in infected controls. Additional studies have been done on the influence of immunization with native MSG on the course of pneumocystis in corticosteroid-treated rats. Injection of MSG into rats before administration of corticosteroids leads to a reduction in the burden of *P. jirovecii* compared to that in controls.[43]

Paracoccidiodes brasiliensis

Paracoccidioidomycosis is a fungal disease that is characterized by a pyogranulomatous tissue reaction. It is found mainly in the tropical and subtropical areas. Gp43 is a major diagnostic antigen for *Paracoccidioides* spp. Functionally, it acts as an adhesin since it binds to laminin-1. CD4 T cells produce IFN-γ on stimulation and may promote fungal clearance. Preliminary data indicate that immunization can reduce the burden of *P. brasiliensis* in the lungs of mice inoculated intratracheally and reduces the severity of inflammation in the lungs.[44]

Recent studies with Saccharomyces

The group at the Santa Clara Valley Medical Centre affiliated with Stanford University had shown protective response in murine models by vaccination with heat-killed *Saccharomyces cerevisiae* against *Aspergillus*, *Coccidioides*, *Cryptococcus* and *Candida.* In this study the killed *Saccharomyces* induced specific immune response—both Th1 and Th2. CD4+ and CD8+ cells were stimulated as well as the antibody against Saccharomyces glucan and mannan. IFN-γ, IL-6 and IL-17A cytokine production was enhanced while alum enhanced the vaccine effect. However, the antigens in the heat-killed preparation inducing the humoral and cellular immunity have not been completely eucidated.[45] Table I lists the various potential fungal vaccines that are being studied.

VACCINATION OF THE IMMUNOCOMPROMISED HOST

Many fungal infections develop in people with a compromised immune status. Vaccines that elicit protective antibodies may be

TABLE I. Fungal vaccines that are presently being evaluated

Fungal pathogen	Vaccine type	Candidate	Target population	Trial phase	Immunity
Candida albicans	Live attenuated	*C. albicans* tet NRG-1 strain[21]	Disseminated candidiasis	Preclinical	Cell-mediated
	Subunit and conjugate	Als protein[18,19]	Disseminated and mucosal candidiasis	Phase -1	Cell-mediated
		Sap2p[3]	Recurrent vulvo-vaginitis	Phase-1	Humoral
		rHyr1p-N[20]	Disseminated candidiasis, neutropenic mice	Preclinical	Humoral
		Surface mannan[23]	Disseminated candidiasis	Preclinical	Humoral
		Enolase[24]	Disseminated candidiasis	Preclinical	Humoral
Aspergillus spp.	Recombinant antigen	Asp f3[27]	Immunosuppressed mice	Preclinical	Cell-mediated
		Asp 16f[28]	Cyclophosphamide treated mice	Preclinical	Cell-mediated
	Antigen pulsed cells	Dendritic cells with fungal RNA[29]		Preclinical	Cell-mediated
Cryptococcus neoformans	Subunit and conjugate	Glucuronoxylomannan capsule[31]	HIV infected	Phase 1	Humoral
		Mannosylated ovalbumin[32]	Immunocompromized individuals	Preclinical	Cell-mediated
Histoplasma capsulatum	Subunit	Hsp60[33]	BALB/c mice	Preclinical	Cell-mediated
	Antigen pulsed cells	Apoptotic phagocytes with heat killed histoplasma[34]		Preclinical	Cell-mediated
Blastomyces dermatitidis	Subunit	Blastomyces adhesin 1 (BAD1)[35]	Immunocompromised mice	Preclinical	Both
Coccidioides immitis	Live attenuated	*C. posadasii* strain[37,38]	Immunocompetent	Preclinical	Cell-mediated
	Subunit	Spherule outer wall (SOW) antigen[39]	Immunocompetent	Phase 1	Cell-mediated
		Antigen 2/Proline-rich antigen (Ag2/PRA)[41]	Immunocompetent	Preclinical	Cell-mediated
Pneumocystis jirovecii	Subunit	p55[42]	Steroid treated rats	Preclinical	Humoral
		Glycoprotein A antigen[43]	Steroid treated rats	Preclinical	Humoral
Paracoccidioides braziliensis	Subunit	Gp43[44]	Immunocompetent mice	Preclinical	Cell-mediated

more useful in these conditions than those that only elicit cell-mediated immunity, because of the relative longevity of circulating antibodies. However, even they would eventually decrease in titres, especially in patients whose immune system is compromised over a long time. The delivery of a vaccine in combination with cytokines which are known to enhance the immune system might be an option. Another approach could be to administer the vaccine along with immunocompetent T or B cells, or dendritic cells that present antigens to T cells can be administered to enhance the immunogenicity of the vaccine.[2]

Adding to the complexity of the situation is the fact that clinicians do not yet have the means to identify immuno-compromised individuals who will develop fungal infections. Although various risk factors are known, not every individual at risk develops a serious fungal infection. Therefore, better methods are needed to identify those at highest risk to determine who would benefit the most from vaccination or a combination of vaccination and immunorestorative therapy.

Universal vaccine for mycotic infections

The experiments involving protective antibody responses against β-1,3-glucan, a glycan that activates complement and is recognized by Dectin-1, suggest that a universal fungal vaccine could be developed. The β-glucans are found in many fungal pathogens and play an immunomodulatory role in various infections. Therefore, β-glucan could be an important vaccine component.[46,47] The inclusion of other oligosaccharides, such as the GXM heptasaccharide epitope and *C. albicans* β-linked mannotriose, could also be included. Additional antigens that can be considered are GXM peptide mimotopes and the LKVIRK epitope of the *C. albicans* Hsp90 antigen.[48]

The choice of the protein carrier for carbohydrate epitopes could further broaden the spectrum of coverage. The use of fungal proteins that act as carriers is likely to induce protective Th1-dependent responses or additional protective antibody responses. For example, two or three carbohydrate moieties could be coupled to a mixture of Hsp60 from *H. capsulatum*, BAD1 from *B. dermatitidis*, and Ag2/PRA protein from *C. immitis.*[49]

CONCLUSIONS

There is a growing need for preventive or therapeutic vaccines to limit the rising incidence of fungal infections. For decades a variety of antigens from both opportunistic and indigenous fungi have been studied in preclinical studies, mainly in murine models. There have been few clinical trials in humans till date. A major reason for the lack of human trials has been the high costs relating to good manufacturing process and production of the antigens, toxicity studies in animals, and high costs for clinical trials in humans. Prototypic antigens have been identified, against which protective immunity can be induced varying from antibody-mediated immune responses to cell-mediated responses or a combination of both. However, concern regarding the safety and

efficacy of fungal vaccines has been raised including safety in immunodeficient hosts, whether they can prevent disseminated disease without affecting the normal microbiota and whether fungal vaccines against agents commonly encountered by humans will result in, or possibly prevent, allergic manifestations. Therefore, further clinical testing should be undertaken to evaluate the efficacy of fungal vaccines in humans. Non-replicating antigens that would protect against a broad range of commonly encountered fungal pathogens remains elusive and the search is on.

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