Fungal vaccines: real progress from real challenges

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Among viral, bacterial, and fungal diseases, the latter are the only branch of infectious diseases without a vaccine for any of their causative agents. This is at odds with a disease burden that remains unabated by conventional chemotherapy and infection control measures. Since most fungal infections occur in immunocompromised patients, the generation of tools relying on host immunity for effectiveness is a notable challenge. Nevertheless, with improved knowledge of the host–fungus relation, and the spectacular advances in genome sequencing, genetic engineering, and proteomics, strong progress in fungal vaccine research is being made. Some vaccines induce the generation of directly fungicidal antibodies; others are protective in animals carrying major risk factors for fungal infections, such as CD4+ T-cell-deficiency or neutropenia. Together with the demonstrated efficacy of various antibodies in passive vaccination approaches, there is growing confidence in the future availability of safe and efficacious immunological tools to combat deadly microbes in a weak host.

Introduction

Vaccines against fungal diseases are gaining ever increasing medical attention, as witnessed by the recent flood of relevant articles, reviews, and commentaries on the subject.[1–28] This renewed interest has mainly been caused by the growing impact of fungal diseases in modern medicine and the largely perceived need to invest in immunological tools to integrate with or replace chemotherapy, therefore minimising antibiotic use and consequent resistance. Another important contributory factor is an increased understanding of the host–fungus relation, which has been fuelled by genomic and proteomic approaches. In this Review, I will discuss the medical need for fungal vaccines, the challenging nature of fungi as vaccine targets, and new approaches in the generation of fungal vaccines and protective antibodies.

The case for fungal vaccines

Recent figures have revealed the alarming impact of fungal infections on human health. Data from older studies[29,30] on patients in health-care institutions have recently been confirmed by reports[31–34] showing that fungal infections rank among the first five causes of infections, with an absolute incidence rate above 1%. The spectrum of fungal pathogens is widening, in parallel with a rise in immunosuppression caused by other medical conditions, including HIV infection, population ageing, and treatments requiring or inducing breakage of cutaneous and mucosal integrity. In particular, *Candida* species have become the fourth most common nosocomial bloodstream isolate in the USA and in most European countries.[32–37] There are clearly defined risk factors for deep-seated mycoses, which are frequent even in non-immunocompromised patients: heavy colonisation with the organism, gastrointestinal and cardiac surgery, long stay in the intensive care unit (ICU), use of broad-spectrum antibiotics, parenteral nutrition, and also simple massive exposure to fungi, as for primary endemic mycoses.[38–40]

Invasive fungal infections are frequent and severe in the settings of haematological malignancies and organ transplant, where they cause substantial mortality. Patients undergoing haematopoietic stem cell transplant appear to be particularly vulnerable to a variety of fungal pathogens, including zygomycetes and *Fusarium* spp, with mortality exceeding 60%.[32,40,41] In a multicentre collaborative study recruiting 11 802 patients in Italy (SEIFEM cohort),[41] moulds—including rare ones such as *Scedosporium* spp, *Acremonium* spp, *Cladosporium* spp, and *Penicillium* spp—were responsible for 346 cases of invasive infection, compared with 192 infections caused by *Candida* spp, *Cryptococcus* spp, and *Trichosporon* spp.

Substantial improvements have been made in fungal infection chemotherapy, with the availability of new azole derivatives and inhibitors of glucan synthase.[42–48] Although the introduction of these new agents may improve the efficacy of antifungal prophylaxis in at-risk patients and provide a valid alternative to old drugs in refractory or resistant cases,[7,49–51] it is not yet clear to what extent the new drugs will affect the overall incidence and mortality caused by fungal disease. This uncertainty is a result of their limited antifungal spectrum, the emergence of new, poorly susceptible filamentous fungi, and the difficulties still encountered in rapid and accurate diagnosis of invasive infection. Furthermore, drug interactions and environmental moulds continue to be challenging aspects of disease control.[39,52] Thus, the mortality rate for invasive candidiasis, one of the most common fungal infections, has remained stable from 1997 to 2003 (at around 0·4 per 100 000 population in the USA[33]), despite the introduction of the new agents, which are almost all effective against *Candida* spp. The above findings underline the urgent need for novel approaches to combat fungal infections, with immunopreventive or immunotherapeutic interventions deserving increased attention.

Challenging vaccine targets

Most fungal diseases pose daunting obstacles to the concept and practice of vaccination, at least in its active immunisation modality. Leaving aside the primary, geographically limited, and low-incidence deep-seated diseases such as coccidiomycosis, histoplasmosis, blastomycosis, and paracoccidioidomycosis, most other widespread illnesses such as aspergillosis, cryptococcosis, and candidiasis (in this last case, with the possible

exception of some forms of mucosal candidiasis) typically occur in the immunocompromised or otherwise debilitated host (table 1 and figure 1). Therefore, the highest impact in terms of incidence and lethality of fungal diseases occurs in patients who are—theoretically—ineligible for active immunisation because of their underlying immunological deficit. This particularly vulnerable status—which so markedly influences a patient's outcome even when infection is promptly diagnosed and effectively treated[53]—is obviously the most influential determinant of vaccine effectiveness. Rather than being protectively immunised, immunocompromised patients might experience aggravation of the immunological disorder following inappropriate immunostimulation by vaccine antigens and adjuvants. Also, in the case of passive vaccination with antibodies, the state of host immunity, particularly its complement and phagocytic assets, is relevant because most antibodies owe their effectiveness to opsonisation and complement fixation. Only efficacy trials with an antigenic formulation that is proved safe and immunogenic in animals will solve this conundrum. Nevertheless, an implicit consensus has emerged in the literature that high numbers of at-risk patients would benefit from a vaccine—preventive or therapeutic—even under conditions of partial immunodeficiency.

Taking invasive aspergillosis as an example, a long list of target populations for vaccination have been described.[12] The list includes: (1) candidate patients for bone marrow transplant, before or after initial engraftment; (2) candidate patients for solid organ

	Biology	Pathogenicity	Disease
Candida spp	Several species, of which *Candida albicans* is the most pathogenic. *C albicans* can grow as both yeast and mycelial forms (hyphae), which are prevalent at 37°C. Pseudohyphae can also be formed. *C albicans* are commensal organisms of the human gastrointestinal tract, with a worldwide distribution.	Extracellular pathogens. Possess well-defined virulence traits such as various adhesins and aspartic proteinase enzymes. Hyphae formation also contributes to virulence in vivo.	Cause superficial infections (skin and various mucosae, particularly vaginal and oral) and deep-seated infections, in nearly all internal organs. Vaginal infection with *Candida* spp is oestrogen-dependent, and probably the most diffuse fungal infection worldwide, affecting around 75% of all women in fertile age at least once.
Cryptococcus spp	*Cryptococcus neoformans* is the only human pathogen. The fungi grow exclusively in the yeast form, can live both intracellularly and extracellularly, and have a worldwide distribution.	*C neoformans* is an extracellular and intracellular pathogen. The fungus has a prominent polysaccharide capsule, which is its major virulence determinant. Melanin also appears to be involved in virulence.	*C neoformans* causes systemic infections, particularly meningoenchephalitis in immunocompromised hosts—eg, HIV-infected patients.
Aspergillus spp	Environmental moulds, with a worldwide distribution, growing as conidia and hyphae. *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus terreus* are the main pathogenic species, which are difficult to control in the hospital environment.	The conidia are the infectious particles, which are transmitted via inhalation. Intramacrophagic differentiation and hyphal growth occurs in infected tissues. There are no well-defined virulence traits.	The most common causal agent of deep-seated infection in haematopoietic stem cell transplanted patients. *Aspergillus* spp can also cause allergic aspergillosis.
Pneumocystis spp	The species that infects human beings is *Pneumocystis jirovecii* (the former *Pneumocystis carinii* is the rat species). The fungi have a complex biological cycle with spores and cystic forms, and a worldwide distribution.	Infection occurs by inhalation of spore forms.	One of the most common opportunistic infections (primary) in HIV-infected patients and patients with neoplasia.
Histoplasma spp	*Histoplasma capsulatum* is the human pathogen. It is a dimorphic fungus, growing as mycelium in the soil and as yeast in vivo. The fungus has a limited geographical distribution in tropical areas, mostly in the USA.	*H capsulatum* is an intracellular pathogen, usually acquired through respiratory infection in immunocompetent host.	Causes primary (pulmonary) endemic mycosis, but also deep-seated pathologies, mainly in immunocompromised hosts.
Blastomyces spp	*Blastomyces dermatidis* is the human pathogen. It is a dimorphic fungus growing as yeast in vivo and as mould in the environment soil (North America).	The protein Bad1 contributes to virulence.	Various clinical forms, ranging from cutaneous to respiratory and systemic.
Coccidioides spp	Two species are human pathogens: *Coccidioides immitis* and *Coccidioides posadasii*. These speces differ in geographical localisation in North, Central, and South America. Both species have identical, complex life cycles. Dimorphic fungi, growing in the soil as saprophytic mycelial forms and spores, with spherules and arthroconidia in vivo.	The infectious forms are the inhaled arthroconida. The tissue-invasive form is the large spherule in which endospores are differentiated.	Causes the so-called "San Joaquin valley fever" a disseminated, lethal infection even in non-immunocompromised patients. Primary infection is an influenza-like illness, with around 10% of patients developing pneumonia and a severe disseminated infection. Certain population groups, for instance Filipinos, appear to be at particularly high risk of developing coccidioidomycosis.
Paracoccidioides spp	The species that infects human beings is *Paracoccidioides brasiliensis*, which is a dimorphic fungus, living as yeast at 37°C in vivo and as hypha in the soil. The fungus is geographically limited to Latin America. Dimorphism appears to be blocked by oestrogens in women of fertile age who are not infected.	No specific virulence attribute is known.	Causes a primary pulmonary infection, with progressive forms and visceral involvements in adults. May cause atypical lymphoproliferative diseases in young patients.

Table 1: **The biological and pathological features of major human pathogenic fungi currently considered as important targets for vaccines**

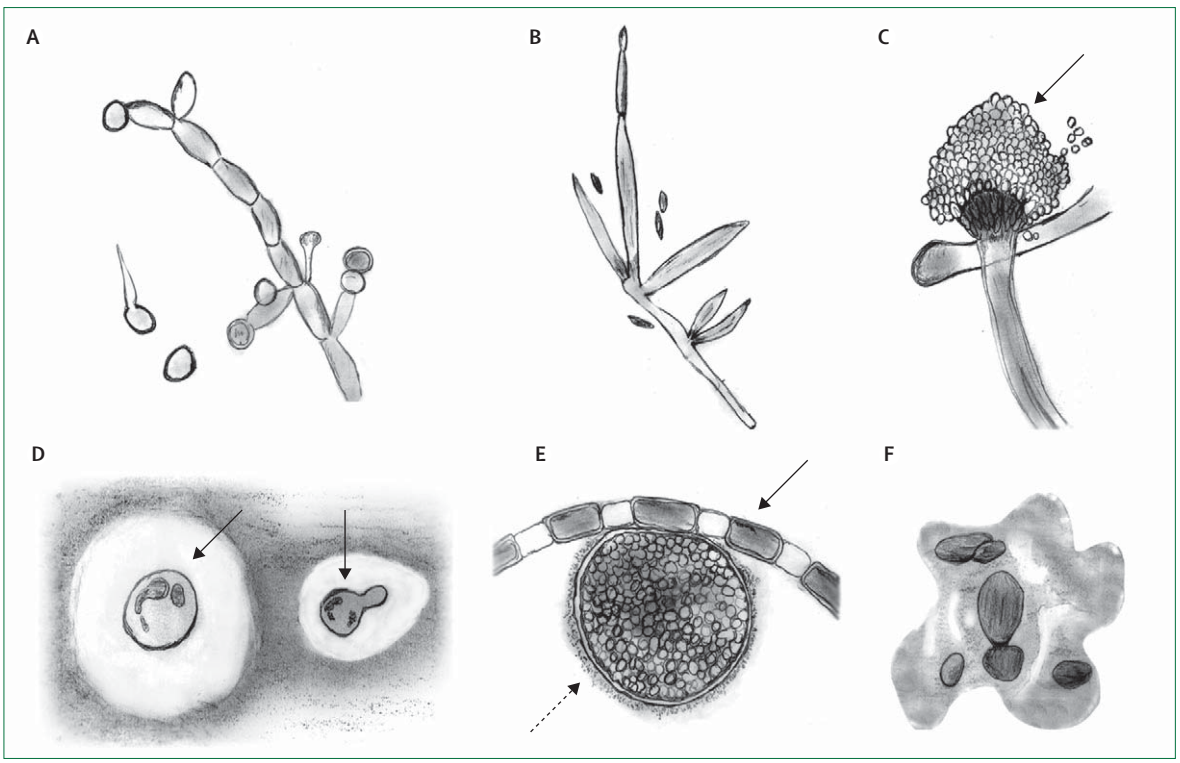

Figure 1: **Schematic drawings of fungi causing human disease**
(A) *Candida albicans*. (B) *Fusarium* spp. (C) *Aspergillus fumigatus* (arrow, conidia). (D) *Cryptococcus neoformans* (arrows, large capsule surrounding the cell). (E) *Coccidioides* spp (single arrow, arthroconidia; dotted arrow, spherule with endospores). (F) *Histoplasma capsulatum*, budding intracellular yeast forms.

transplant, who could be suitably immunised as to develop effective immunity while waiting for the transplant; (3) patients with acute myeloid leukaemia or solid tumours even after receiving initial cytostatic chemotherapy, taking into consideration that immune responses are usually compromised several weeks from the initiation of therapy; and (4) patients with inflammatory bowel disease, before the use of immunosuppressive corticosteroids and tumour necrosis factor α (TNFα) blockers. Other vaccine targets are patients undergoing deep surgery, particularly gastrointestinal or cardiac, or recovering from surgery in the ICU. Such patients are at-risk of invasive candidiasis or aspergillosis even when not profoundly immunodepressed.[7,12,46] In particular, critically ill patients admitted to the ICU while still immunocompetent are at substantial risk of aspergillosis and other opportunistic fungal infections during the stage of abnormal immune function, if not frank immunoparalysis, which follows septic shock and endotoxin tolerance.[54]

As well as immunosuppression or host debilitation, other challenges in the development of vaccines include the human commensal nature of fungi (eg, *Candida* spp) and the capacity of fungi to establish clinical latency (eg, *Cryptococcus* spp, *Coccidioides* spp). There are no data to suggest that candida commensalism benefits the host, although this suspicion needs to be considered when addressing anticandida vaccination. In the case of latency or chronic infection bouts (as for recurrent vaginal candidiasis), disease occurs upon reactivation, and this might require special formulations of therapeutic rather than prophylactic vaccines. Finally, the occurrence of allergic diseases, as in the case of aspergillosis, could complicate the generation of anti-aspergillus vaccines. A thorough knowledge of the type of immune responses that help the host clear or resist infecting fungi is essential for the development of an efficacious vaccine.

Immune responses against fungi

As for other human pathogens, a close collaboration between innate and adaptive immunity is crucial for antifungal defence. The protective role of well-known factors of innate immunity, such as mechanical barriers and phagocytes (eg, polymorphonuclear cells and macrophages), is indirectly but extensively illustrated by the existence of classic risk factors for opportunistic fungal infections, including indwelling central venous catheters, neutropenia, and use of corticosteroids. Complement and other humoral factors of innate immunity, such as antifungal peptides and the mannose-binding lectin,[55,56] have also been shown to have a role.

Recent studies have highlighted the crucial role of dendritic cells[57–61] in linking innate to adaptive immunity and organising the nature and extent of antifungal defence (figure 2). As antigen presenting cells, dendritic cells process the antigen and present its epitopes to T cells

within the context of MHC class I or II molecules. Pattern recognition receptors (PRRs) on dendritic cells interact with surface-exposed, highly conserved molecules (the so-called pathogen-associated molecular patterns [PAMPs]), such as mannoproteins and β-glucan in fungi (figure 3), and transduce signals for early inflammatory and non-specific responses. PRRs that have been intensely studied include Toll-like receptors (TLRs), complement receptor 3, mannose receptor, Fcγ receptor, and Dectin-1.[62] TLR2 and TLR4 have been shown to be particularly involved in antifungal responses, possibly mediating cooperative or counter-regulatory signals together with other PRRs.[26,63–66] PAMP-PRR interaction triggers a complex cascade of intracellular signalling that ultimately leads to the production of cytokines such as interleukin 12 and interleukin 23, activation and differentiation of naive T cells into antigen-specific CD4+ T helper (Th) or CD8+ T cells, and expression of antifungal activity by the humoral and cellular arms of adaptive immunity.

Until now, generation of novel vaccine adjuvants has taken place on an empirical ground.[63–67] However, identification of the various fungal PAMPs and their mechanisms of interaction with PRRs is now hastening adjuvant generation on a rational ground, and might lead to the replacement of aluminium salts. One example is the CpG oligodeoxynucleotide adjuvant for experimental vaccination against *Aspergillus* spp.[67] Surprisingly, chloroquine, an old antimalarial drug, has recently been suggested as a potent adjuvant because of its marked cross-priming ability and capacity to activate CD8+ T cells, a property of remarkable importance in an antifungal vaccine.[68] Some of the immunogenicity of the glycoconjugate vaccine β-glucan (laminarin)-diphtheria toxoid might be caused by the well-known β-glucan adjuvanticity,[13,62] or even by the toxoid itself. In fact, bacterial toxins are among the most powerful vaccine adjuvants in experimental models and some of them are in clinical trials for use in human beings. Interestingly, CpG and β-glucan bind to different TLRs (TLR9 and TLR2, respectively), thus indicating that adjuvants may use more than one signalling mechanism to help vaccine effectiveness.[62,65–67]

Cell-mediated immunity is commonly believed to be the primary defence against fungal diseases, as indirectly witnessed by clinical observations in patients with innate or acquired defective cell-mediated immunity, including HIV infection.[4,7,37] This theory has been supported by immunological approaches in mice with genetic deletion of T-cell subsets and cytokines, which showed increased susceptibility to fungal infections (including candidiasis and histoplasmosis), depending on the type of T-cell defect.[7,26] Figure 2 summarises the main aspects of antifungal cell-mediated immunity. Cytokines, such as interferon γ and TNFα produced by CD4+ Th1 lymphocytes, are strong activators of phagocytic cells, which are capable of killing or arresting fungal growth. Additionally, natural killer lymphocytes, CD4+ T cells, and CD8+ T cells can exert direct cytotoxicity against some fungi upon activation by interleukin 2 in vitro,[69] although the in-vivo relevance of this phenomenon is still unknown.

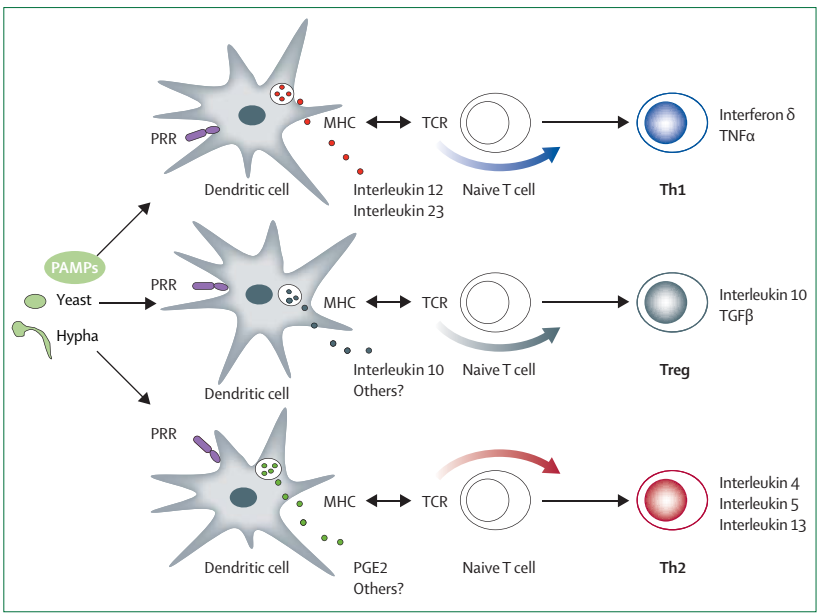

Figure 2: **Role of dendritic cells in inducing/regulating adaptive immunity**
Note the antigen processing with membrane association with MHC class II (vesicles in dendritic cells of top and bottom rows) and the intracytoplasmic processing pathway associated with MHC class I (vesicles in dendritic cell middle row). See text for details. PAMP=pathogen-associated molecular pattern. PRR=pattern recognition receptor. TCR=T-cell receptor. Th1=T-helper cell type 1. Th2=T-helper cell type 2. TGFβ=transforming growth factor β. TNFα=tumour necrosis factor α. Treg=regulatory T cell. PGE2=prostaglandin E2.

Since interferon γ and TNFα are potentially dangerous inflammatory cytokines, a well-balanced immune response usually requires the generation of anti-inflammatory and regulatory cytokines such as interleukin 10 and interleukin 4 by CD4+ T cells, Th2 lymphocytes, T regulatory cells, and Th1 cells.[70] Notably, Th2 cytokines, such as interleukin 10 and interleukin 4, are usually non-protective in animal models of fungal infection.[4,26,57] In addition to Th1 and Th2 effectors, two other T-cell subsets (Th0 and Th17) have been detected. The relevance of these cell subsets in antifungal immunity is currently being studied. Particularly intriguing is the role for Th17 cells (which produce interleukin 17 and interleukin 23) in the generation of *Candida albicans*-specific human memory T cells[71] and in promoting susceptibility to fungal infection.[72]

Important points to consider in antifungal immunity and its relevance to vaccination are that usually fungi display only moderate virulence (table 1),[73,74] and antifungal immune responses are usually redundant. Although almost all pathogenic fungi have mechanisms to evade or intoxicate immune responses,[75–80] residual immunity may still be beneficial to the host. Examples illustrating this point are that CD8+ T-cell activation can replace CD4+ T cells in the induction of protection against histoplasmosis in a CD4+ T-cell-deficient mouse model, as well as the

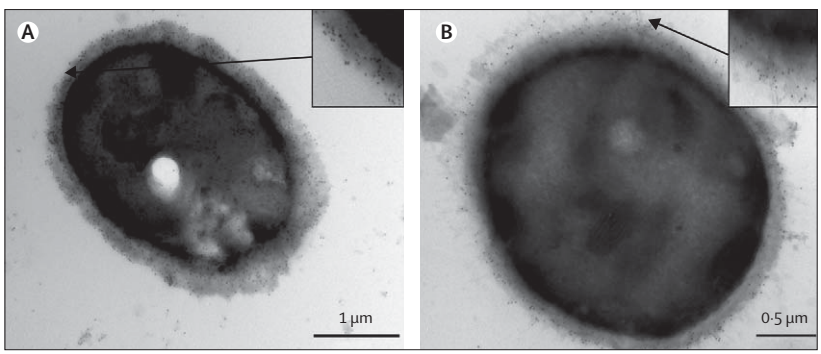

Figure 3: **Electron micrographs of *Candida albicans* in cryofixed specimen**
The cell wall localisation of (A) β-glucan and (B) mannan are shown. Insets are larger magnification pictures to show the gold labels. For immunogold labelling, two IgM monoclonal antibodies were used, one (1E12) specific for β-glucan and one (mAb AF1) specific for a β-mannoside sequence within *C albicans* mannan.[2,13]

direct anticandidal and anticryptococcal activity of cytotoxic CD8+ T cells.[81–83] Finally, there is no need for a vaccine to be fungus-eradicating: neutralisation of adhesins and enzymes or other low-penetrance virulence traits may be sufficient to avoid disease.[13]

Antibodies and passive vaccination

Clinical inferences and the results of some experimental models, particularly in endemic primary mycosis, have clearly confirmed the main protective role of cell-mediated immunity.[4,7] However, protective immune sera, mucosal antibodies, some murine and human monoclonal antibodies, and genetically engineered antibody fragments have all shown remarkable efficacy in fighting fungi.[4,13,14] These observations have special relevance for vaccination, particularly in partly or totally immunocompromised patients. In principle, antibodies can be induced by vaccination in at-risk patients before they become immunocompromised. Furthermore, because of the longevity of IgG (weeks to months depending on the IgG subclass), antibodies might persist with a protective titre even during prolonged immunosuppression. There is some experimental evidence that vaccination before immunosuppression could work for many fungal infections, including *Pneumocystis jirovecii* pneumonia.[81,84] Admittedly, the above approach is hardly achievable with vaccines exclusively eliciting antifungal T cells, pro-inflammatory cytokines, and activating macrophages or neutrophils, all events of much shorter persistence.

Importantly, highly specific humanised or human antibodies, in a variety of different formats, are becoming available to fight infections,[85–87] as has been seen in the field of tumour and chronic autoimmune diseases—eg, palivizumab for the treatment of respiratory syncytial virus. Monoclonal human recombinant antibodies and their fragments have recently been generated and used in experimental fungal infection[87–101] (table 2). One monoclonal recombinant antibody is nearing regulatory approval (Mycograb; antibody against heat shock protein [HSP] 90),[22] whereas others are still in the pipeline.[87] Several engineered antibodies without an Fc (fragment, crystallisable) region have been described with proven antifungal efficacy,[88,89,97] suggesting that they can work efficiently even in the absence of phagocytic effector cells or complement. Other protective murine and human monoclonal antibodies against *Candida* spp and *Cryptococcus* spp have been shown to activate the classic pathway and the deposition of complement products on the cell surface in a specific way,[90,99] although the true role of complement activation for passive protection by human anticryptococcal antibodies remains to be defined.[100] So far, no consistent evidence of a therapeutic effect of passive vaccination has been provided for infections caused by *Histoplasma* spp, *Coccidioides* spp, *Blastomyces* spp, and *Paracoccidioides* spp. However, antibodies to a cell surface component were protective against *Histoplasma capsulatum*.[101]

Because of quantity restrictions, high cost, and limited effectiveness inherent in a pure antibody approach, it is likely that antibody therapy will be used in combination with antifungal agents, as suggested by Larsen and colleagues[20] for the anti-glucuronoxylomannan antibody, and applied by Pachl and colleagues[23] in the case of Mycograb.

Antibody-based immunotherapeutic or even preventive antifungal strategies require careful consideration of antibody specificity, affinity, and isotype. In fact, identical antibody specificity but different isotype may reverse a protective antibody into a non-protective or even disease-enhancing one.[19,100] Host status is also crucial. Immunocompromised patients may lack or have inefficient Fc-dependent effector functions (phagocytes, complement). Thus, antibodies that neutralise virulence traits, particularly adhesins, or antibodies that can directly inhibit fungal growth or even kill the fungus should be the preferred treatments in these patients. Various examples of the fungistatic or fungicidal capacity of some antifungal antibodies have been reported.[88,92,94,95,97] The most useful antibodies are probably anti-β-glucan antibodies, since in principle they can affect all human pathogens that share this viability-critical and immutable cell wall component.[2,13,122,123] Importantly, antibodies that are non-fungicidal in their native state may be rendered fungicidal by labelling with a radiation emitter, which is already used for anti-cancer therapy.[124–126] This technique is now being explored for antifungal immunotherapy: initial testing of an anticapsular glucuronoxylomannan-directed antibody bound to ^{213}Bi has been done in experimental cryptococcosis.[125] One effect of the radiolabelled antibody was to decrease the size of the cryptococcal capsule,[125] the main virulence trait of this fungus. Curiously, this effect is also evident in vivo with the anti-β-glucan antibody mAb2G8.[2,125,127]

Specific vaccines and antibodies

Table 3 summarises some of the antifungal vaccines that have successfully provided both active and passive

	Antigens	Underlying immunity	References
Whole cells and Ill-defined cell extracts			
Candidiasis	Strain CA2, live-attenuated	T-helper 1, cell-mediated immunity	24,102
	Ribosomal cell fraction	Antibodies and cell-mediated immunity	10,103
	Inactivated whole cells	Undefined	104
Blastomycosis	Strain BAD1, live attenuated	T-helper 1, cell-mediated immunity	4,7,57,105
Coccidioidomycosis	Inactivated spherules	T-helper 1, cell-mediated immunity	3,15,106
Aspergillosis	Inactivated, conidia	Undefined	8
	Live attenuated conidia	Undefined	8
Histoplasmosis	Ribosomal vaccine	Undefined	4,6,7
DNA			
Coccidioidomycosis	More than one gene	Undefined	107
Paracoccidioidomycosis	gp43 gene	T-helper 1, T-helper 2, cell-mediated immunity	108
Pneumocystosis	Kexin gene	Cell-mediated immunity and antibodies	81
Antigen-pulsed cells and T cells			
Candidiasis	Dendritic cell loaded with candida antigens	Cell-mediated immunity, T-helper 1	24,25,109
Aspergillus antigen-specific T cells	Dendritic cell loaded with aspergillus antigens	Cell-mediated immunity, T-helper 1	24,25,110
Subunit and glycoconjucates			
Candidiasis	Agglutinin-like sequences	Cell-mediated immunity	4,111,112
	Secreted aspartic proteinase2	Anti-Sap2 antibodies	113
	65 kDa mannoprotein	Adhesin-neutralising antibodies	114
	β-1,3-glucan	Growth-inhibitory and cytocidal antibodies	2,13
	β-1,2-mannosides	Antibodies (opsonophagocytic; possibly adherence-blocking)	4,99,115
Cryptococcosis	Capsular polysaccharide	Various mechanisms	1,5,116
	Glucuronoxylomannan-conjugated vaccine	Unknown, possibly antibodies	1,5,116
Aspergillosis	Aspergillus fumigatus antigens	Cell-mediated immunity	8,67,110,117
	β-1,3-glucan	Growth inhibitors, antibodies	2,13,66
Coccidioidomycosis	Antigen2	Cell-mediated immunity, T-helper 1	3,15
	β-1,3-glucosyl transferases	Undefined	3,15
	Chimeric polyprotein	Undefined	3,15,118
Pneumocystosis	P55 protein (major surface glycoprotein)	Undefined, possibly antibodies	119
	Kexin protease	Cell-mediated immunity and antibodies	81
Paracoccidioidomycosis	43 kDa glycoprotein	Cell-mediated immunity and antibodies	108
Idiotypes and mimotopes			
Candidiasis	Killer-toxin neutralising mAb KT4	Fungicidal antibodies	96,120,121
Aspergillosis	Killer-toxin neutralising mAb KT4	Fungicidal antibodies	93
Cryptococcosis	Glucuronoxylomannan-peptide mimotopes	Antibodies modulating cell-mediated immunity	5
Antibodies			
Candidiasis	Mycograb, anti-Hsp90 peptide†	Unknown	21–23
	Anti-β-1,3-glucan mAb 2G8	Growth-inhibitory	2,13
	mAb C7 (stess mannoprotein)	Candidacidal	95
	Single chain fragment variable of anti-idiotypic antibodies	Candidacidal antibodies	93
	Anti-mannan mAb C6	Opsonophagocytic	99,115
	Anti-glycosyl mAb	Candidacidal	92
	Anti-Sap2 and anti-MP65 domain antibodies	Enzyme and adhesion-neutralising	89
Cryptococcosis	Single chain fragment variable	Inhibits glucan synthase	97
	Anti-glucuronoxylomannan 18B7-mAb (murine)	Opsonophagocytic	20
	Anti-glucuronoxylomannan IgG2 (human)	Opsonophagocytic	100
Histoplasmosis	Antibody against histone-like protein	Undefined	101

mAb=monoclonal antibody. Hsp90=heat shock protein 90. *Selected for existence of in-vivo protection data. †Under clinical trials.

Table 2: **Major fungal vaccines for active and passive immunisation***

Antibody format	Disease	Setting
Polyclonal	Candidiasis (invasive and mucosal)	Experimental
	Cryptococcosis	Experimental
	Pneumocystosis	Experimental
Monoclonal murine	Candidiasis (invasive and mucosal)	Experimental
	Cryptococcosis	Experimental and clinical
	Aspergillosis	Experimental
	Pneumocystosis	Experimental
Monoclonal human	Candidiasis (invasive)	Experimental and clinical
	Cryptococcosis	Experimental
Single-chain, fragment variable	Candidiasis (invasive and mucosal)	Experimental
	Cryptococcosis	Experimental
Antibody domains	Candidiasis (mucosal)	Experimental
Antibody-derived peptides	Candidiasis (invasive and mucosal)	Experimental
	Cryptococcosis	Experimental

Table 3: **Antibodies used for experimental and clinical passive vaccination**

immunisation. These vaccines have all shown consistent activity in at least one experimental model of fungal disease. The few preparations that have undergone clinical trials are dealt with below.

Nearly all types of chemical and antigenic formulation, including antigen-encoding DNA, have been considered for active vaccination and nearly all major fungal pathogens have been addressed.[102–121,128–133] With present-day regulatory hurdles, it is quite unlikely that vaccines based on complex and ill-defined antigenic mixtures will be approved, even if they are shown to be immunogenic and protective in the preclinical setting. This is chiefly because of the difficulties in ensuring batch consistency and standardisation of the product.[104,128] Advances in whole genome sequencing and proteomics[87,129,130] are now making it possible to know most—if not the whole set—of fungal proteins; this knowledge allows for selection of a discrete number of antigens to test for protection, exactly as it has been done for bacterial vaccines (eg, group B meningococcus, in an approach called reverse vaccinology).[130] This approach couples with antigen reactivity with immune sera or T cells from patients recovering from disease, and bioinformatic algorithms (in-silico prediction), in identifying novel vaccine candidates. Recent examples of the application of this "antigenome" approach[87] have been provided by Thomas and co-workers[129] for anticandida vaccine and by Tarcha and colleagues[118] for a multivalent vaccine against *Coccidioides* spp.

The results of these novel approaches to candidate vaccine antigens make it unjustified or unrealistic to further pursue antigenic extracts or even inactivated whole-cell vaccines, which may be affected by safety issues.[15] Attenuated fungal cells are potently protective vaccines in animal models (eg, the CA2 strain of *C albicans*),[26,102] but could not be used in immunocompromised patients. However, studies of experimental blastomycosis and histoplasmosis suggest that attenuated strains of agents of endemic, primary mycoses can be efficacious in normal, non-immunocompromised hosts.[4,7]

Subunit vaccines remain the most researched types of fungal vaccines and are most likely to result in an approvable product. They consist of one or more purified proteins (usually recombinant in nature), or one or more polysaccharides, rendered sufficiently immunogenic through conjugation with a protein carrier (mostly bacterial toxoids).[2,115,116] Polysaccharide subunit vaccines include those based on original approaches such as the peptide mimotopes[5,9] and yeast killer toxin-neutralising antibody.[96,120,121] Some subunit vaccines are based on antigens that are common in different fungal species[111,112] or even genera,[2,13] raising the possibility of immunisation against several fungi with a single antigenic formulation (the so-called universal antifungal vaccine).[2] Examples include the HSP60[4,6] and β-glucan[2,13] immunogens. The idiotypic vaccine based on an antibody that neutralises a killer toxin,[96,120] the anticandida vaccine based on Als proteins,[111,112] and Mycograb[23] also belong to this category. The spectrum of fungal targets may be so broad as to encompass (theoretically) all fungal pathogens, as for the β-glucan-conjugate vaccine. One advantage of β-glucan-conjugate vaccine is that β-glucan is a fungal molecule essential for cell wall construction and fungus survival, thus no counter-selection is likely. Recent studies have shown that β-glucan can be a target for protective antibodies against *Cryptococcus neoformans* and *Pneumocystis carinii*.[127,131] The concept of a universal vaccine, as promoted by fungal vaccinologists, could be extended to bacterial vaccines since different bacteria share common or similar targets, for instance, the peptidoglycan and the lipopolysaccharides.

Experimental antifungal vaccination with DNA plasmids encoding one or more protein antigens has also been attempted.[81,107,108,133] DNA vaccines stimulate both CD4 and CD8+ T cells through MHC class I and MHC class II antigen presentation pathways, with concomitant activation of phagocytic/cytotoxic effectors and humoral responses. This type of vaccine could also be protective in a CD4+ T-cell-deficient host.[81] Priming with DNA and boosting with the recombinant protein and/or modifying the properties of antigen-presenting cells[132] are procedures that could be immunogenic and potentially protective against intracellular fungi. However, despite theoretical promise, the production of DNA vaccines that are safe, immunogenic, and protective in human beings is proving to be difficult, and no DNA vaccine has yet been approved for human use.

Two interesting approaches are the use of fungus antigen-primed dendritic cells or fungus-specific T-cell clones. These cells can be generated ex vivo and suitably infused in the host, avoiding induction or potentiation of graft-versus-host disease, or damaging stem cell graft.

Romani and colleagues[24,25,109,110] have described the benefits of these approaches. Identification of fungus-protective antigens for selective priming-activation of dendritic cells and generation of highly focused selective T-cell clones could improve this approach.

Since protection against most fungal diseases is provided by cellular effectors, passive vaccination has mainly been tested in diseases where more extensive and pioneering work on the protective role of antibodies has been done—namely candidiasis and cryptococcosis. Data indicating the feasibility of passive vaccination against pneumocystosis have also been published.[119]

Clinical trials of active and passive vaccination

There is no fungal vaccine approved or currently undergoing advanced clinical trials for active immunisation in human beings. However, several vaccine manufacturers have fungal antigens under development as candidate vaccines. Two vaccine formulations have undergone limited phase I and phase II trials: the first against vulvovaginal candidiasis by a candida ribosomal preparation,[103] and the second against cryptococcosis by the tetanus toxoid-conjugate of the capsular polysaccharide glucuronoxylomannan.[9,134] A more extensive efficacy trial was done with a vaccine against coccidioidomycosis.[15,103,135] Overall, the results of these trials offered valid data on immunogenicity and, in the case of vulvovaginal candidiasis, the vaccine also showed some partial protection, but did not encourage further progress. In particular, a trial in which about 3000 volunteers were injected with a killed vaccine made from formalin-treated *Coccidioides* spp spherules showed that the vaccine was unacceptably toxic, of low immunogenicity, and inefficacious.[106,135] Furthermore, experimental evidence that the glucuronoxylomannan-conjugate vaccine against cryptococcosis could elicit both protective and disease-enhancing antibodies in mice instilled a severe hurdle to the extension of clinical trials of glucuronoxylomannan-based vaccines.

Nonetheless, these investigations generated valid reagents and information to pursue the use of anti-glucuronoxylomannan antibody for passive vaccination against cryptococcosis. One such murine monoclonal antibody (mAb 18B7) has recently undergone a phase I trial of dose-finding, safety, and pharmacokinetics for prospective use as adjunctive therapy against cryptoccoccal meningitis.[20,134,136] The investigation was undertaken in HIV-infected patients who had been successfully treated for cryptococcal meningitis. Antibody doses ranging from 0·01 mg/kg to 2 mg/kg of bodyweight were used as a single infusion, and doses up to 1 mg/kg were safe or only mildly toxic. Higher antibody doses were toxic and, in one patient, severely toxic. The study also showed that the mAb 18B7 had a serum half-life of approximately 53 h, and was undetectable in the cerebrospinal fluid of all patients. The investigators concluded that continued investigation of mAb 18B7 at a maximum single dose of 1·0 mg/kg was necessary. Since some of the toxicity could be related to the heterologous nature of the antibody, it would make sense to try to humanise it by genetic engineering before further trials. However, it has recently been reported that a human anti-glucuronoxylomannan IgG1 is disease-enhancing in mice,[100] urging further caution.

The results of a randomised, blinded, multicentre trial that compared treatment of invasive candidiasis with liposomal amphotericin B only with amphotericin B plus Mycograb in 117 patients have recently been published.[22,23] In an intention-to-treat analysis of the two therapeutic arms, the combined treatment was shown to be superior to chemotherapy alone in the overall clinical and mycological response. The antibody was well tolerated, with the possible exception of hypertension episodes in some patients following the initial dose.[23,27]

Conclusions

The increased awareness of the medical threat represented by fungal diseases and the persistent inability of chemotherapy to reduce their incidence and lethality have renewed interest in the search for vaccines against human pathogenic fungi. Novel approaches for developing fungal vaccines, particularly genome sequencing and proteomics, promise a real breakthrough in this area. Similarly, knowledge of the immune response against fungi, as well as the practice of selecting adjuvants that stimulate a balanced innate immunity, will also become important factors in choosing vaccine formulations for clinical trials.

The clinical use of directly fungicidal or growth-inhibitory antibodies (with or without radio-labelling) is offering some innovative approaches to other branches of infectious diseases. Some antibody formulations have been generated that appear to kill the fungus by inhibiting the glucan synthase enzymes,[97] acting like the echinocandin-derived antimycotics.[43] Passive vaccination with these "antibiotic antibodies"[96] could be a breakthrough therapy in the setting of the immunocompromised host. A caveat here is the selection of the right antibody isotype, in view of the existence of protective and disease-enhancing antibodies of the same antigenic specificity against cell-surface polysaccharides.

Search strategy and selection criteria

Data for this Review were identified by searches of Medline up to June, 2007, for relevant articles in English language. Searches of the author's own files were also done. For the section on clinical trials, the EBM Cochrane Central Register of Controlled Trials database and public US government files were consulted. Papers on vaccines and antibodies were selected if they included consistent data from at least one established in-vivo model of fungal infection. Priority was given to articles with some information on the mechanism of protection. Abstracts and meeting reports were not considered.

More research and clinical trials are likely to favour the application of cytokine and immune cell-based therapy in antimycotic-refractory fungal pathologies, and these applications could synergise with low-dose antifungal chemotherapy and antibody therapy.[137] Despite financial barriers,[138] there is growing confidence that vaccines and other fungus-fighting immunological tools will become clinically available in the near future.

Conflicts of interest

I am the co-owner of two patents on vaccines and antibodies whose licensing rights have been purchased by Chiron Novartis (Siena, Italy). I have also received a grant from Pevion Biotech-Crucell (Bern, Switzerland) for research on a mucosal anticandida vaccine.

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