Recent progress in vaccines against fungal diseases

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Diseases caused by fungi are increasingly impacting the health of the human population and now account for a large fraction of infectious disease complications in individuals with impaired immunity or breached tissue defenses. Antifungal therapy is often of limited effectiveness in these patients, resulting into treatment failures, chronic infections and unacceptable rates of mortality, morbidity and their associated costs. Consequently there is a real medical need for new treatments and preventive measures to combat fungal diseases and, toward this goal, safe and efficacious vaccines would constitute major progress. After decades of complacency and neglect of this critically important field of research, remarkable progress has been made in recent years. A number of highly immunogenic and protective vaccine formulations in preclinical setting have been developed, and at least two have undergone Phase 1 clinical trials as preventive and/or therapeutic tools against candidiasis.

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Medical need and vaccine drivers

After the first decade of the third millennium, or more than two centuries since the first safe and efficacious vaccine (smallpox vaccine) was delivered to humankind, and now approaching half a century since the declaration of smallpox eradication, and after more than a dozen severe lethal bacterial and viral diseases have been kept at bay by vaccination, there is still no vaccine against any fungal disease, including those endemically affecting people in vast areas of the earth. Major obstacles that have hindered, and still continue to have an impact on, the development of fungal vaccines have been discussed elsewhere by ourselves and others [1,2,3•,4,5], and these

include the lack of good quality vaccine formulations and the absence of mass market appeal, with the latter being complicated by the high cost of clinical investigations. However, this situation is rapidly changing by increasing medical need and the attention of both investigators and industry representatives has begun to focus on the need for vaccine against major fungal pathogens. Consequently we are now on the threshold of clinical development for some vaccines against fungal pathogens.

Major drivers of the rather sharp change in perspective regarding antifungal vaccines are considered in Box 1. It is difficult to weight the importance of any single factor for the shift in emphasis but it is likely that a combination of biotechnological progress leading to the generation of well-characterized, highly immunogenic, stable and standardized fungal antigens on one hand and the recognition of our current inability to treat most fungal diseases successfully and at acceptable cost, on the other hand, are the major determinants of the recent progress. The vaccine industry now sees fungal vaccines very differently than in the past, both as a consequence of medical need and as a result of the development of novel ideas by some vaccine pioneers about the role of vaccines in the population, realizing the goals of protecting most vulnerable subjects because of age or underlying disease, and generating vaccines not only to prevent lethal diseases but also to improve quality of life [6,7].

In this latter context, a relevant example is provided by the experience with chronic and recurrent vulvovaginal candidiasis (RVVC) [8]. This is certainly not a life-threatening illness but can have a devastating effect on the quality of life of millions of women worldwide. Although exact numbers for the incidence and prevalence of this disease are difficult to obtain because RVVC is not a reportable disease, it is generally thought that approximately two thirds of all women in fertile age have experienced at least one acute attack of vaginal candidiasis in their lifetime and that 50% have multiple distinct episodes. Importantly, roughly 4–5% of these women develop RVVC (meaning >3 episodes per year of vaginal inflammation). RVVC is very difficult to control and may never be definitely cured before women enter the menopause. Thus, in regards to RVVC, we are dealing with millions of cases per year, as also witnessed by the huge number of medications sold annually worldwide. In recent internet-based self-assessment studies, the rate of RVVC in countries such as US, UK, France, Italy and Spain was reported to be as high as 9% (Foxmann, personal communication, manuscript submitted). *Candida albicans* is the cause of >90% RVVC cases and

Box 1 Major drivers of current interest in promoting research on, and developing, fungal vaccines

Availability of safe, highly immunogenic and protective fungal antigens in several, independent investigations in animal models.

Emergence of rather strong, unmet medical needs concerning both the number of subjects affected by fungal infections and the numerous categories of underlying medical conditions associated with increased susceptibility to fungal disease. Morbidity and mortality particularly high (up to nearly 50% of attributed mortality) in some settings of fungal infections, particularly invasive aspergillosis and candidemia, despite optimal available chemotherapy.

Rising confidence for the ability of vaccine preparations to elicit protective immunity unbiased by pre-existing fungal immunity arising from natural infection or commensalism (particularly for candidiasis).

Market potential for some 'therapeutic' vaccines such as those against recurrent vulvovaginal candidiasis: emergence of the concept of vaccine-to-improve-quality of life.

Public health and industry interest in widening the spectrum of preventive and therapeutic vaccines to cover even non-lethal fungal diseases, assuring protection in vulnerable, aged people with underlying medical conditions:

Scientific progress in an area where novel immunological paradigms can emerge: immune responses in commensalism/frequent exposure/opportunism by eukaryotic infectious agents (see the case for Th17 cells and antibodies).

women with this chronic inflammatory syndrome has become a main target population for a therapeutic anti-candida vaccine (see below).

In summary, it now appears that a sort of virtuous circle has been generated as a result of a confluence of disparate events that has created an environment propitious for vaccine development. Factors that have contributed to this situation include creative competition among various research groups from different disciplines including industrial partners, some of which have now given high priority to fungal vaccine development. This is demonstrated by the number of novel fungal components for vaccine development, immune mechanisms and preclinical studies, making the pair with the rather high numbers of patents dealing with *Candida* vaccines [9]. Of course, judgment is needed to select the most promising vaccines for clinical investigations from a plethora of potential choices, some of which are made by exceedingly complex, uncharacterized antigenic mixtures, while others have been tested in unreliable animal models with doubtful predictive efficacy and still others with no evidence for induction of adaptive immunity and memory responses, and/or mechanisms underlying vaccine efficacy. If we also add to this complexity the issues of adjuvancy and good quality manufacturing (see below), very few of the candidate vaccines meet all basic requisites for clinical trials.

Concerning the vast array of fungal vaccines candidates and their immunological basis several reviews have been recently published and the readers are invited to consult those sources for more detailed information [1,2,3•]. Particularly, this invitation should be met by those who wish to get an insight into the progress of vaccines against North American and South American endemic mycoses whose commercial realization is proving to be remarkably difficult for logistic and marketing aspects despite a record of high quality biochemical and immunological investigations in these fields [3•]. Instead, we wish to highlight here recent progress in some vaccines against opportunistic fungal infections and discuss both some changes in perspective for the target subjects of these vaccines and the underlying immunological paradigms which, in some cases, raise important immunological issues. Particular emphasis will be given to those vaccines which are known to be under clinical trial.

Who can or must be vaccinated?

With the possible exception of vaccines against endemic fungal diseases, such as coccidioidomycosis, paracoccidioidomycosis and histoplasmosis, as well as vaccines against chronic, RVVC (see below), fungal vaccines should primarily be conceived as tools to prevent or help to cure fungal diseases in immunocompromised or otherwise debilitated hosts, since fungal diseases have an extraordinary high mortality and morbidity in this group and consequently there a real medical need for a fungal vaccine. However, there are severe obstacles to the implementation of such a strategy. Many immunocompromised subjects who are prone to develop aggressive fungal infections such as aspergillosis and deep-seated candidiasis are those with hematologic disorders or cancer, which need to be treated with transplant and/or immunosuppressive, anticancer therapy soon after diagnosis. Consequently, the vaccine development for this group poses a problem of leaving too narrow a window to consider vaccination and/or initiate an appropriate schedule of vaccination. In addition, despite several claims that residual, effective immune responses could effectively deal with the pathogen even after deletion or loss of major groups of antifungal defensive cells such as phagocytes and CD4T helper cells [1,3•,10,11] it remains uncertain whether persistent protection could be obtained in these subjects because of their underlying immune defect. Few studies have addressed whether adoptive vaccination by transfer of dendritic cells in these subjects is a realistic option [12].

On the other hand, accurate epidemiologic research has identified a large set of individuals who are at risk of invasive fungal infection, and particularly candidemia and aspergillosis, who cannot be defined as immunodepressed and who may be able to mount appropriate, persistently protective immunity by canonical vaccination approaches. Although this distinction must be assumed with some caution since, for instance, patients undergoing deep surgery will certainly experience a transient phase of immune impairment [13], there are doubtless several

Table 1

Major subunit vaccine candidates against opportunistic fungal infections.

Disease	Vaccine	Nature of protective immunity	References
Candidiasis	β-mannan-peptide or protein conjugates	Opsonic, antibody-mediated	[15••,19]
	HyR1	Antibodies neutralizing *Candida* evasion from neutrophil killing	[20]
	Recombinant Als 3* proteins	Th17–Th1 activity (Abs as surrogate markers or predictors of protection)	[21,43,49]
	Recombinant Sap2* proteins	Antibodies neutralizing Sap activity (enzyme, adhesion and/or others)	[16•]
	Laminarin-CRM197 conjugate	Anti-beta-glucan Abs with direct anti-*Candida* activity	[33•,44,45]
Cryptococcosis	Laminarin-CRM197 conjugate	Antibodies affecting capsule size and function	[34]
	GXM conjugate	Opsonic anti-capsular Antibodies	[17]
	Peptide mimotopes		[17,23]
Aspergillosis	Laminarin-CRM197 conjugate	Unknown: possible direct antifungal activity of antibodies	[44]
	AspF antigens	CD4 T cells (antibodies surrogate or predictors of protection)	[39]
	Cell wall glucanase Crf1	CD4 Th1 cells	[47]

The asterisk denotes the two vaccines currently under clinical trial.

pathologies and/or medical intervention which place individuals with no obvious immune deficit at risk of life-threatening fungal infection. Dominant among these categories are subjects with a breach in their cutaneous and mucosal defenses, particularly intestinal, and bearing permanent central venous catheters, or simply having a long hospital stay, particularly in the intensive care setting. Among subjects in this category, candidemia has become a real public health problem [14]. Hence vaccines against opportunistic fungi will probably be generated and primarily used in one or more of these subjects categories.

Issues about antigenic composition and formulation: the critical role of adjuvants

As for all other vaccines, the proposed fungal vaccines cover a wide range of different composition ranging from whole-inactivated fungal cells and virulence-attenuated mutant organism to subunit vaccines on the basis of single, recombinant protein or peptide fragment or glycoconjugates [15••,16•,17,18–27]. Whole inactivated organisms have the disadvantage of a having a complex chemical composition that poses serious problems with regards standardization and safety. Inactivated cells also elicit weaker immune responses than live vaccines. In contrast, virulence-attenuated mutants are usually one of the best immunogens to achieve specific protection but have the drawback of limited use for immunosuppressed or otherwise debilitated subject who represent theoretically a vast category among those who could benefit of a fungal vaccine, since even attenuated variants can sometime cause disease in individuals with impaired immunity. On the other hand, subunit vaccines (Table 1), which may be the best choice with regards to manufacturing, standardization, and safety, hence easier access to clinical trial, are poorly immunogenic and usually require to be administered with an adjuvant to induce a long-lasting protective immunity. While this is no problem in

experimental animal models, where the classical Freund adjuvant and companions have largely and profitably employed, there is a scarcity of good adjuvants suitable for use in clinical practice. Furthermore, there is little experience with fungal vaccines in humans, and some failure of aluminum salts [18], which are the landmark of human-compatible adjuvants. This has led to the use of oil-in-water mixtures, for instance squalene-based MF59, which have been advocated to broaden the nature of the epitopes recognized including those of low antigenicity, by mechanisms which are still under intense study [18,28]. An alternative to these adjuvants is the use of liposomes, virosomes, inert nanoparticles, PAMP-derived components and other bioengineered preparations [29,30]. One of the two anticandidal vaccines in clinical trial (see below) uses a virosomal platform as adjuvant/carrier whereby the antigen component of the vaccine is stably linked to influenza virus-derived particles embedded in a liposomal formulation. The virosomal formulation conferred high immunogenicity to a recombinant protein with intrinsically low immunogenicity [31].

## Immunological basis of fungal vaccines

One of the most interesting, and intriguing aspect of fungal vaccines lies on the apparent existence of dual immunological mechanisms in achieving protection. As shown in Table 1, the main mechanisms which have been advocated are a Th1-based and/or Th17-based response, or antibody-mediated immunity. Although the different mechanisms can indeed cooperate for the final protective outcome, the distinction remains substantial both for vaccine formulation and for establishing correlates/surrogates of protection in clinical trials. In particular, the T cell-based mechanisms mediate protection indirectly, that is, promoting an inflammatory response with recruitment of soluble (antimicrobial peptides, cytokines, chemokines) and cellular (macrophages, neutrophils)

effectors which ultimately are responsible for the elimination or control of the invading fungal cells at the site of infection [32•]. In contrast, antibodies can mediate protection not only by the classical opsonophagocytosis and complement activation, which ultimately also rely on phagocyte numbers and activation state, but also by direct neutralization of factors such as adhesins or enzymes which are a critical step for infection, or by inhibiting fungal escape from host immunity, or fungal growth or even directly killing the fungus [15••,33•,34]. Recently, specific antibodies to *Cryptococcus neoformans* were shown to directly modulate fungal metabolism opening yet another mechanism by which humoral immunity can potentially alter the outcome of fungal infection [35•]. These mechanisms could prove of particular importance in vaccines aimed at protecting prospective immunocompromised host with defective cellular effectors.

Th17 cells, directly and/or by promotion of, or collaborating with, Th1 responses (Figure 1), have been advocated to be responsible for protection mediated by some vaccines against candidiasis (the Als3 vaccine) but mostly against aspergillosis, and endemic mycoses ([36••,37–39]; Table 1). In addition, this mechanism has a corresponding counterpart in the natural history of fungal infections where DNA polymorphisms affecting receptors and cytokines critical for Th1 and Th17 responses seem to predispose to various forms of mucosal candidiasis and invasive aspergillosis (reviewed in [32•]). A vaccine on the basis of eliciting an exclusive Th1/Th17 response as effector cells would be a novelty in the field of human vaccines, since almost all of existing vaccines mediate protection through neutralizing antibodies. A potential drawback in this kind of immunoprotective mechanism is the requirement for an intact capacity to recruit and activate phagocytes, which can be either deficient in immunocompromized host setting or can be themselves the cause of the inflammatory disease as for chronic recurrent vaginal candidiasis and other mucosal infections [8,40] Overall, the role of Th17, their interaction with the other CD4 cell subsets, and antibodies in fungal vaccines remain a subject of intense research interest. With regards to *C. albicans*, we note the report that specific T lymphocytes produce both IFN-g and IL-17, that is, the cytokine signatures of Th1 and Th17 cells respectively [41]. Of interest is also that in some vaccines relying on Th17 elicitation for protection, specific antibodies may nonetheless predict the achievement of the protective state [36••,42,43].

More in line with vaccine historical achievements are those on the basis of antibody neutralizing one or more of critical factors for fungal infection, yet this field has long been neglected by fungal vaccinologists because of the apparent lack of evidence of antibody involvement in the natural history of fungal infections, with the possible exception of anti-capsular polysaccharide antibodies in

Figure 1
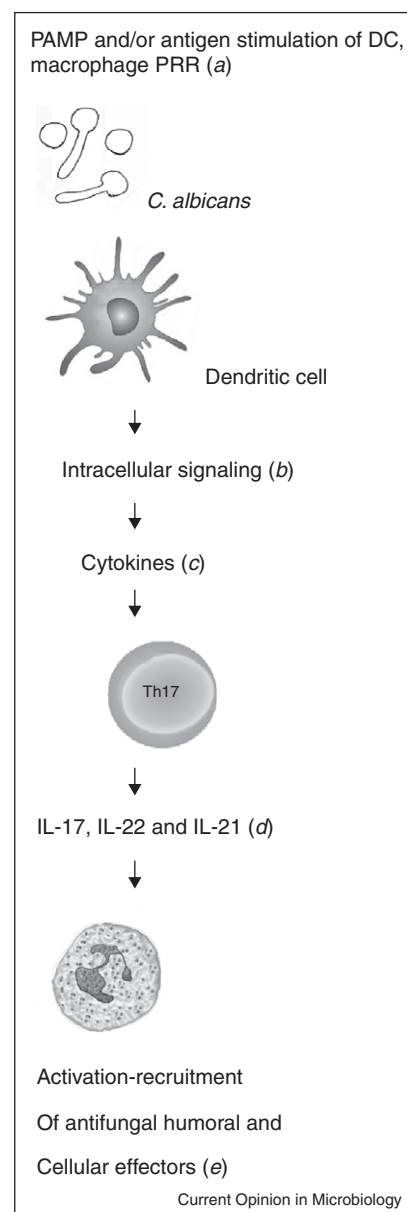
Highlights on Th17 cells as a major cellular platform for antifungal defense and vaccination. **(a)** PRR, pattern recognition receptors such as Toll-like receptors, Dectin-1, mannose receptors and others. They can differ between DC and macrophages and be differently involved in recognition of different fungal PAMP (Beta-glucan, mannoproteins, GXM, *etc*.) expressed on fungal surface. **(b)** and **(c)** Various mechanisms, often interrelated and involving transcription factors, inflammation activation and cytokines such as type 1-IFN. IL-10, IL-12, IL-23 and others depending on signaling cascade. IL-23 is particularly critical for Th17 expansion. **(d)** IL-17 isoforms IL-17 A and IL-17F. **(e)** Antifungal defensins, chemokines, inflammatory cytokines, PMN (neutrophils).

cryptococcal vaccines. With the noted exception of the vaccines against endemic mycoses, there is now undisputable evidence that the majority of the vaccines proposed against infection by major fungal pathogens such as

Box 2 Major differences between the two vaccine antigens of *C. albicans* under clinical trial

Als 3	Sap 2
A cell surface, GPI protein member of Als adhesin family without known enzymatic activity: interacts with various members of host integrin family	A major member of secreted aspartic proteinase family of *C. albicans* with direct or indirect adhesin activity. May interact and hydrolyze various host immunologically relevant proteins such as complement antibodies and epithelial structural proteins such as E-cadherin
Involved in biofilm formation	No apparent role in biofilm (other members of Sap family may be involved)
Modulates iron acquisition by hyphae	A classical metabolic role as proteinase of *C. albicans*, both in yeast and hyphae
Candida-colonized subjects have both CMI and Ab responses against Als 3	Low or no levels of antibodies and CMI responses in *Candida*-colonized subjects, likely because of Sap2 low immunogenicity in its natural form
Vaccine induces protection through elicitation of Th1 and Th17 cells, then the cohort of antifungal humoral and cellular factors acting locally and promoting inflammation	Vaccine induces protection through elicitation of neutralizing antibody at vaginal level
Vaccine target: candidiasis systemic and mucosal	Vaccine target: recurrent vulvovaginal candidiasis

C. albicans and *Cryptococcus neoformans* owe their experimental protection to antibodies as formally shown by passive serum transfer and use of monoclonal antibodies (Table 1). Interestingly enough, both *C. albicans* and *Cryptococcus neoformans* are the pathogenic fungi in which there is also more compelling evidence for the existence of true aggressive factors such as capsule, adhesins and virulence enzymes to quote just a few, which mediate a degree of fungal virulence and contribute to the disease. Antibodies neutralizing these virulence factors are obvious mediators of vaccine-induced protection, particularly those antibodies which are not major components of fungus-pre-existing immunity in humans. In line with this reasoning, a glycoconjugate 'universal' vaccine eliciting antibodies to beta 1–3 glucan has been shown to confer significant protection experimental models of aspergillosis, candidiasis and cryptococcosis [33•,44,45] New technological improvements in the induction of adaptive immunity by glycoconjugate vaccines could further enhance the wide-spectrum protective efficacy of this and other antifungal glycoconjugates [46]. A recent report confirms the possibility of using *Candida* and *Aspergillus* cross-reactive antigens for vaccine protection [47].

Vaccines in clinical trial

Two fungal vaccines are currently in Phase 1 clinical trial, both designed to protect against *Candida* infections, particularly chronic recurrent candidal vaginitis (Box 2). A first vaccine is on the basis of a N-terminus portion of a recombinant Als3 with alum as adjuvant (Nova Digm, USA). This vaccine has proven protective in various experimental models of *Candida* infections, both mucosal and systemic. In mice, protection appears to be mediated by the cooperative activity of Th1 and Th17 cells, and be independent on antibodies and B cells. Antibodies are indeed elicited by vaccination and have been proposed as surrogate markers of effective

vaccination or even 'predictors' of protective state [42]. In this regards susceptibility to *Cryptococcus neoformans* has been associated with deficits in IgM memory B cells [48]. For *C. albicans*, normal human subjects are primed against Als proteins, and have both cell-mediated and anti-Als3 serum antibodies [49]. A report on satisfactory safety and immunogenicity profile from Phase 1 clinical trial of the Als3 vaccine (single dose) has been posted on the web (www.novadigm.net). In parallel studies, Als3 vaccine has been claimed to exert protection also against experimental infection by *Staphylococcus aureus* through a mechanism that may involve antigenic mimicry [38,43].

A second vaccine that is progressing in Phase 1 clinical trial is a virosomal formulation of a N-terminus truncated, recombinant secretory aspartyl proteinase (Sap2) of *C. albicans* (PEV7 by Pevion Biotech, Switzerland). On the basis of strong evidence for the critical role of this enzyme and homologous Sap in human and experimental candidal vaginitis [16•,50], this vaccine is designed primarily for therapeutic use against RVVC. The vaccine appears to confer protection by Sap-neutralizing antibodies which are absent or at very low levels in serum or vaginal fluid of healthy *Candida* colonized women. Initial safety as well as immunogenicity data of PEV7 in women is strongly encouraging (www.pevion.com). Interestingly, data suggesting for a protective role of Th17 cells in a murine model of candidal vaginitis have recently been published [51].

Conclusions

Years ago, David Stevens called to the arms to enlist the immune system in the fight against fungal infections [52]. Vaccines represent a gold standard among the tools derived from the various arms of the immune system, hence their generation and use is the best way to respond to that call. Obstacles remain for the commercial development of

vaccines against medically important fungi and these are largely financial, but at least for some infections in some categories of patients a vaccine will probably be available in the next future. This will finally fill the gap noticed in the field of anti-infectious vaccines and will represent a fundamental public health achievement in a medical area still affected by substantial, morbidity, mortality and high cost of cure.

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