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Recent Progress in Vaccines against Fungal Diseases

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Abstract

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.

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 (1-5), 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

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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*). *C. albicans* is the cause of >90% RVVC cases and 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 (1-3). Particularly, this invitation should be met by those who wish to get an insight into the progress of vaccines against North- 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 CD4 T 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 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 based on single, recombinant protein or peptide fragment or glycoconjugates (15-28). 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 (19), 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 (19,29). An alternative to these adjuvants is the use of liposomes, virosomes, inert nanoparticles, PAMP-derived components and other bioengineered preparations (30,31). 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 (18).

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 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, i.e. 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 *C. 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 (36-39; 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 based on 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 immunocompromised 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- γ and IL-17, i.e. 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 based on 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 cryptococcal vaccines. With the noted exception of the vaccines against endemic mycoses, there is now undisputable evidence. However, there is now undisputable evidence that the majority of the vaccines proposed against infection by major fungal pathogens such as *Candida 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 *C. 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-preexisting 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 based on a N-terminus portion of a recombinant Als3 with alum as adjuvant (Nova Digm, US). 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 *C. 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 (16,18,50), 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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References

1. Cutler JE, Deepe GS, Klein D. Advances in combating fungal diseases: vaccines on the threshold. *Nat Rev Microbiol.* 2007; 5:13–28. [PubMed: 17160002]
2. Cassone A. Fungal vaccines: real progress from real challenge. *Lancet Infect Dis.* 2008; 8:114–124. [PubMed: 18222162]
- *3. Spellberg B. Vaccines for invasive fungal infections. *F1000 Medicine Reports.* 2011; 3:13. Excellent summary of the state of the art for several vaccines against fungi. [PubMed: 21876719]
4. Stevens DA, Clemons KV, Liu M. Developing a vaccine against aspergillosis. *Med Mycol.* 2010; 49(Suppl 1):S170–6. [PubMed: 20608783]
5. Datta K, Pirofski LA. Towards a vaccine for *Cryptococcus neoformans*: principles and caveats. *FEMS Yeast Res.* 2006; 6:525–36. [PubMed: 16696648]
6. Rappuoli R, Mandl CW, Black S, De Gregorio E. Vaccines for the twenty-first century society. *Nat Rev Immunol.* 2011; 11:865–872. [PubMed: 22051890]
7. Moxon ER, Siegrist CA. The next decade of vaccines: societal and scientific challenges. *Lancet.* 2011; 378:348–359. [PubMed: 21664685]
8. Fidel PL Jr, Cutler JE. Prospects for development of a vaccine to prevent and control vaginal candidiasis. *Curr Infect Dis Rep.* 2011; 13:102–1077. [PubMed: 21308461]
9. Wang SJ. *Candida* vaccines development from point view of US patent application. *Hum Vaccin.* 2011 Nov; 7(11):1165–71. [PubMed: 22048114]
10. Wuthrich M, Filutowicz HI, Warner T, Deepe GS, Klein BS. Vaccine immunity to pathogenic fungi overcomes the requirement for CD4 help in exogenous antigen presentation to CD8+ T cells: implications for vaccine development in immune-deficient hosts. *J Exp Med.* 2003; 197:1405–16. [PubMed: 12782709]
11. Koh AY, Köhler JR, Cogshall KT, Van Rooijen N, Pier GB. Mucosal damage and neutropenia are required for *Candida albicans* dissemination. *PLoS Pathog.* 2008; 4:e35. [PubMed: 18282097]
12. Bozza S, Perruccio K, Montagnoli C, Gaziano R, Bellocchio S, Burchielli E, Nkwanyuo G, Pitzurra L, Velardi A, Romani L. A dendritic cell vaccine against invasive aspergillosis in allogeneic hematopoietic transplantation. *Blood.* 2003; 102:3807–14. [PubMed: 12791648]
13. Cardinale F, Chinellato I, Caimmi S, Peroni DG, Franceschini F, Miraglia Del Giudice M, Bernardini R. Perioperative period: immunological modifications. *Int J Immunopathol Pharmacol.* 2011:S3–12. [PubMed: 22014920]

14. Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. *Crit Rev Microbiol.* 2010; 36:1–53. [PubMed: 20088682]
- **15. Xin H, Cutler JE. Vaccine and monoclonal antibody that enhance mouse resistance to candidiasis. *Clin Vaccine Immunol.* 2011; 18:1656–67. Report describes a new approach to synthesizing conjugate vaccines against *C. albicans* and provides additional evidence for the protective role of antibody-mediated immunity. [PubMed: 21832099]
- *16. Sandini S, La Valle R, Deaglio S, Malavasi F, Cassone A, De Bernardis F. A highly immunogenic recombinant and truncated protein of the secreted aspartic proteases family (rSap2t) of *Candida albicans* as a mucosal anticandidal vaccine. *FEMS Immunol Med Microbiol.* 2011; 62:215–24. Report describes a new protein-based vaccine against *C. albicans* that mediates protection by eliciting antibodies that neutralize a fungal protease. [PubMed: 21535228]
17. Ostrowski-Zeichner L, Casadevall A, Galgiani JN, Odds FC, Rex JH. An insight into the antifungal pipeline: selected new molecules and beyond. *Nat rev Drug Discov.* 2010
- *18. De Bernardis, Flavia; Amacker, Mario; Arancia, Silvia; Sandini, Silvia; Gremion, Christel; Moser, Christian; Zurbriggen, Rinaldo; Cassone, Antonio. A virosomal vaccine against candidal vaginitis: immunogenicity, efficacy and safety profile in animal models. *Vaccine.* submitted.
19. Bromuro C, Romano M, Chiani P, Berti F, Tontini M, Proietti D, Mori E, Torosantucci A, Costantino P, Rappuoli R, Cassone A. Beta-glucan-CRM197 conjugates as candidates antifungal vaccines. *Vaccine.* 2010; 28:2615–23. [PubMed: 20096763]
20. Xin H, Dziadek S, Bundle DR, Cutler JE. Synthetic glycopeptide vaccines combining beta-mannan and peptide epitopes induce protection against candidiasis. *Proc Natl Acad Sci U S A.* 2008; 105:13526–31. [PubMed: 18725625]
21. Luo G, Ibrahim AS, Spellberg B, Nobile CJ, Mitchell AP, Fu Y. *Candida albicans* Hyr1p confers resistance to neutrophil killing and is a potential vaccine target. *J Infect Dis.* 2010; 201:1718–28. [PubMed: 20415594]
22. Spellberg BJ, Ibrahim AS, Avanesian V, Fu Y, Myers C, Phan QT, Filler SG, Yeaman MR, Edwards JE. Efficacy of the anti-*Candida* rAls3p-N or rAls1p-N vaccines against disseminated and mucosal candidiasis. *J Infect Dis.* 2006; 194:256–60. [PubMed: 16779733]
23. Ito JI, Lyons JM, Hong TB, Tamae D, Liu YK, Wilczynski SP, Kalkum M. Vaccinations with recombinant variants of *Aspergillus fumigatus* allergen Asp f 3 protect mice against invasive aspergillosis. *Infect Immun.* 2006; 74:5075–84. [PubMed: 16926399]
24. Datta K, Lees A, Pirofski LA. Therapeutic efficacy of a conjugate vaccine containing a peptide mimotope of cryptococcal capsular polysaccharide glucuronoxylomannan. *Clin Vaccine Immunol.* 2008; 15:1176–87. [PubMed: 18524882]
25. Saville SP, Lazzell AJ, Chaturvedi A, Monteagudo C, Lopez-Ribot JL. Efficacy of a genetically engineered *Candida albicans* tet-NRG1 strain as an experimental live attenuated vaccine against hematogenously disseminated candidiasis. *Clin Vaccine Immunol.* 2009; 16:430–432. [PubMed: 19144791]
26. Li WQ, Hu XC, Zhang X, Ge Y, Zhao S, Hu Y, Ashman RS. Immunization with the glycolytic enolase confers effective protection against *Candida albicans* infection in mice. *Vaccine.* 2011; 29:5526–5533. [PubMed: 21645574]
27. Liu M, Capilla J, Johansen ME, Alvarado D, Martinez M, Chen V, Clemons KV, Stevens DA. *Saccharomyces* as a vaccine against systemic aspergillosis: ‘the friend of man’ a friend again? *J Med Microbiol.* 2011; 60:1423–32. [PubMed: 21825307]
28. Cutler JE, Corti M, Lambert P, Ferris M, Xin H. Horizontal Transmission of *Candida albicans* and evidence for a vaccine response in mice colonized with the fungus. *PLOS One.* 2011:6.
29. Seubert A, et al. Adjuvanticity of the oil-in-water emulsion MF59 is independent of Nlrp3 inflammasome but requires the adaptor protein MyD88. *Proc Natl Acad Sci US.* 2011; 108:11169–11174.
30. Ferwerda G, Netea MC, Joosten LA, Van der Meer JWM, Romani L, Kullberg BJ. The role of Toll-like receptors and C-type lectins for vaccination against *Candida albicans*. *Vaccine.* 2010; 28:614–622. [PubMed: 19887129]
31. Chauban A, Swaleha Z, Ahmad N, Farazzudin M, Vasco A, Abida M, Mohammad O. Escheriosomes mediated cytosolic delivery of *Candida albicans* proteins induces enhanced

- cytotoxic T lymphocyte response and protective immunity. *Vaccine*. 2011; 29:5424–5433. [PubMed: 21645572]
- *32. Romani L. Immunity to fungal Infections. *Nat Rev Immunol*. 2011; 11:275–288. A detailed review of antifungal immunity focusing on innate immunity and immune homeostasis. [PubMed: 21394104]
- *33. Torosantucci A, Chiani P, Bromuro C, De Bernardis F, Palma AS, Liu Y, Mignogna G, Maras B, Colone M, Stringaro A, Zamboni S, Feizi T, Cassone A. Protection by anti-beta-glucan antibodies is associated with restricted beta-1,3 glucan binding specificity and inhibition of fungal growth and adherence. *PLoS One*. 2009; 4(4):e5392. A conjugate vaccine that elicits antibodies to beta glucans is protective against *C. albicans*, *A. fumigatus*, and *C. neoformans* thus establishing the precedent that vaccines that elicit broad immunity against fungi are feasible and possible. [PubMed: 19399183]
34. Rachini A, Pietrella D, Lupo P, Torosantucci A, Chiani P, Bromuro C, Proietti C, Bistoni F, Cassone A, Vecchiarelli A. An anti-beta-glucan monoclonal antibody inhibits growth and capsule formation of *Cryptococcus neoformans* in vitro and exerts therapeutic, anticryptococcal activity in vivo. *Infect Immun*. 2007; 75:5085–94. [PubMed: 17606600]
- *35. McClelland EE, Nicola AM, Prados-Rosales R, Casadevall A. Ab binding alters gene expression in *Cryptococcus neoformans* and directly modulates fungal metabolism. *J Clin Invest*. 2010 Apr 1; 120:1355–1361. This paper reports that antibody binding to a fungal structure can alter fungal cell metabolism thus indicating a new mechanism by which antibody-mediated immunity can modulate microbial infection. [PubMed: 20335660]
- **36. Wolrich M, Gern B, Hung CY, Ersland K, Rocco N, Pick-Jacobs J, Galles J, Filutowicz H, Warner T, Evans M, Cole G, Klein B. vaccine-induced protection against 3 systemic mycoses endemic to North America requires Th17 in mice. *J Clin Invest*. 2011; 121:554–568. This paper describes a comprehensive study including *H. capsulatum*, *C. immitis*, and *B. dermatitidis* provides strong evidence that some antifungal vaccines must elicit Th17 immune responses for protective efficacy. [PubMed: 21206087]
37. Hung CY, Gonzales A, Wuthrich M, Klein BS, Cole GT. Vaccine immunity to coccidioidomycosis occurs by early activation of three signal pathways of T helper cell responses (Th1, Th2 and Th17). *Infect Immun*. 2011; 79:4511–4522. [PubMed: 21859851]
38. Lin L, Ibrahim AS, Xu X, Farber JM, Avanesian V, Baquir B, Fu Y, French SW, Edwards JE, Spellberg B. Th1-Th17 cells mediate protective adaptive immunity against *Staphylococcus aureus* and *Candida albicans* infection in mice. *PLoS Pathog*. 2009; 5:e1000703. [PubMed: 20041174]
39. Diaz-Arevalo D, Bagramyan K, Hong TB, Ito JI, Kalkum M. CD4+ T cells mediate the protective effect of the recombinant Asp f3-based anti-aspergillosis vaccine. *Infect Immun*. 2011; 79:266.
40. Zelante T, De Luca A, Bonifazi P, Montagnoli C, Bozza S, Moretti S, Belladonna ML, Vacca C, Conte C, Mosci P, Bistoni F, Puccetti P, Kastelein RA, Kopf M, Romani L. IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. *Eur J Immunol*. 2007 Oct; 37(10):2695–706. [PubMed: 17899546]
41. Fenoglio D, Poggi A, Catellani S, Battaglia F, Ferrera A, Seti M, Murdaca G, Zocchi MR. Vdelta1 T lymphocytes producing IFN γ patients and IL-17 are expanded in HIV-infected and respond to *Candida albicans*. *Blood*. 2009; 113:6611–6618. [PubMed: 19395673]
42. Spellberg B, Ibrahim AS, Lin L, Avanesian V, Fu Y, Lipke P, Otoo H, Ho T, Edwards JE. Antibody titer threshold predicts anti-candidal vaccine efficacy even though the mechanism of protection is induction of cell-mediated immunity. *J Infect Dis*. 2008; 197:957–71. [PubMed: 18429348]
43. Lin L, Ibrahim AS, Baquir B, Avanesian V, Fu Y, Spellberg B. Immunological surrogate marker of rAls3p-N vaccine-induced protection against *Staphylococcus aureus*. *FEMS Immunol Med Microbiol*. 2009; 55:293–5. [PubMed: 19159425]
44. Torosantucci A, Bromuro C, Chiani P, De Bernardis F, Berti F, Galli C, Norelli F, Bellucci C, Polonelli L, Costantino P, Rappuoli R, Cassone A. A novel glyco-conjugate vaccine against fungal pathogens. *J Exp Med*. 2005; 202:597–606. [PubMed: 16147975]
45. Chiani P, Bromuro C, Cassone A, Torosantucci A. Anti-beta-glucan antibodies in healthy human subjects. *Vaccine*. 2009; 27:2513–519. [PubMed: 19368794]

46. Auci FY, Li X, Tanji M, Kasper DA. Mechanisms of glycoconjugate vaccine activation of the adaptive immune system and its implications for vaccine design. *Nat Medicine*. 2011; 17:1602–1609.
47. Stuehler C, Khanna N, Bozza S, Zelante T, Moretti S, Kruhm M, Lurati S, Conrad B, Worschech E, Stevanovi S, Krappmann S, Einsele H, Latgé JP, Loeffler J, Romani L, Topp MS. Cross-protective TH1 immunity against *Aspergillus fumigatus* and *Candida albicans*. *Blood*. 2011; 117:5881–5891. [PubMed: 21441461]
48. Subramaniam K, Metzger B, Hanau LH, Guh A, Rucker L, Badri S, Pirofski LA. IgM(+) memory B cell expression predicts HIV-associated cryptococcosis status. *J Infect Dis*. 2009; 200:244–51. [PubMed: 19527168]
49. Bequin B, Lin L, Ibrahim AS, Avanesian V, Tu A, Edwards J, Spellberg B. Immunological reactivity of blood from healthy humans to the rAls3p-N vaccine. *J Infect Dis*. 2010; 201:475–479.
50. De Bernardis F, Arancia S, Morelli L, Hube B, Sanglard D, Schäfer W, Cassone A. Evidence that members of the secretory aspartyl proteinase gene family, in particular SAP2, are virulence factors for *Candida vaginitis*. *J Infect Dis*. 1999 Jan. 179:201–208. [PubMed: 9841840]
51. Pietrella D, Rachini A, Pines M, Pandey N, Mosci P, Bistoni F, d'Enfert C, Vecchiarelli A. Th17 cells and IL-17 in protective immunity to vaginal candidiasis. *PLoS One*. 2011; 6(7):e22770. [PubMed: 21818387]
52. Stevens DA. Vaccinate against aspergillosis! A call to arms of the immune system. *Clin Infect Dis*. 2004; 38:1131–1136. [PubMed: 15095219]
53. Moser C, Amacker M, Zurbriggen R. Influenza virosomes as a vaccine adjuvant and carrier system. *Expert Rev Vaccines*. 2011; 10:437–46. [PubMed: 21506642]

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 (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)

Box 2Major 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 <i>C. albicans</i> 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 <i>C. albicans</i> , 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 <i>Candida</i> -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 |

Highlights

- We review recent progress in fungal vaccine development
- The climate for fungal vaccine development is increasingly favorable
- At least two vaccines against fungal diseases are in clinical trials
- Experimental work suggests that it may be possible to generate broad fungal vaccines against several important pathogens

PAMP and/or antigen stimulation of DC, macrophage PRR (a)

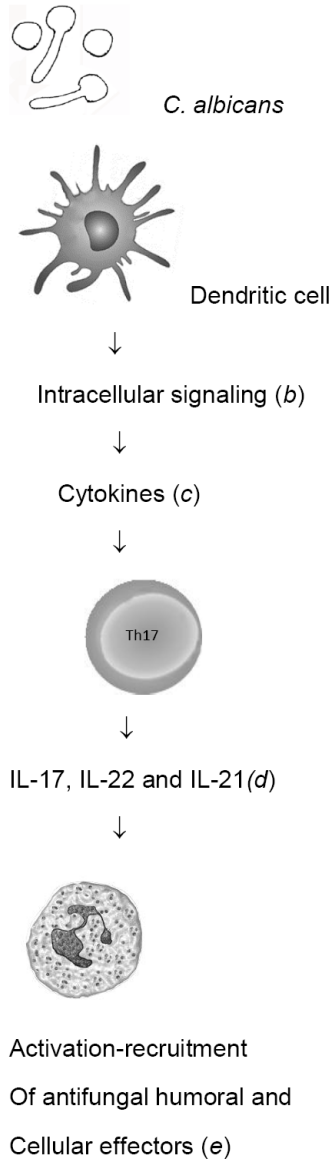


Figure 1. Highlights on Th17 cells as a major cellular platform for antifungal defense and vaccination

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)

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 | 15,20 |
| | HyR1 | Antibodies neutralizing Candida evasion from neutrophil killing | 21 |
| | Recombinant Als 3* proteins. | Th17-Th1 activity (Abs as surrogate markers or predictors of protection) | 22,43,49 |
| | Recombinant Sap2* proteins | Antibodies neutralizing Sap activity (enzyme, adhesion and/or others) | 16,18 |
| | 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 peptide mimotopes | Opsonic anti-capsular Antibodies | 17 17,24 |
| 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