Candida albicans Vaccines

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Summary: 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 has been made. Some vaccines induce the generation of directly antifungal antibodies; others are protective in animals carrying major risk factors for fungal infections. Together with 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.

Key Words: Vaccines, Candida albicans, Immunity.

Introduction

Candida albicans is the most common cause of opportunistic fungal diseases in human (Schaberg et al., 1991). In the immunocompromised patients, disseminated candidiasis is a serious disease which often results in death, even in patients treated with antifungal agents such as amphotericin B (Anttila et al., 1994; Komshian et al., 1989) (Table 2). Difficulties associated with both the diagnosis of disseminated candidiasis and treatment of the disease by conventional means argue in favor of pursuing the development of preventive strategies and alternative forms of treatment (Berenguer et al., 1993; Reboli, 1993). In experimental animal models of candidiasis, optimal antifungal protection has been achieved by vaccination with an attenuated low-virulence strain or after spontaneous recovery from the initial infection (Bistoni et al., 1986; Cassone et al., 1995; Fidel et al., 1998; Romani et al., 1992). Since candidiasis is especially prevalent among immunocompromised subjects, however the use of inactivated whole cell or subunit vaccines should be, in principle, a safer and more convenient approach. Vaccines against fungal diseases are gaining ever increasing medical attention due to development of new virulent strains and their impacts thereafter. This review aims to discuss
the medical need for candida vaccine, the challenging nature of candida as vaccine targets, and new approaches in the generation of candida vaccines and protective antibodies.

The case of fungal vaccines
Available figures support the alarming impact of fungal infections on human health. Fungal infections rank among the first five causes of infections, with an absolute incidence rate above 1% (Wisplinghoff et al., 2004; Nucci et al., 2005). The spectrum of fungal pathogens is widening in parallel with a rise in immunosuppression caused by other factors including HIV infection, population ageing, and treatments requiring or inducing breakage of cutaneous and mucosal integrity. Candida species, in particular have become the fourth most common nosocomial bloodstream isolate in the USA and in most European countries (Pfaller et al., 2007; Sims et al., 2005; Morris et al., 2006; Wenzel et al., 2005). Invasive fungal infections are frequent and severe in the settings of hematological malignancies and organ transplant, where they cause substantial mortality. Patients undergoing hematopoietic stem cell transplant appear to be particularly vulnerable to a variety of fungal pathogens with mortality exceeding 60% (Nucci et al., 2005; Safdar, 2006; Pagano et al., 2006). Improvements have been made in fungal infection chemotherapy with the availability of newazole-derivatives and inhibitors of glucan synthase (Cappelletty et al., 2007; Polak, 2003; Wengard, 2007). 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 (Deep et al., 2005; Cornely et al., 2007; Segal et al., 2007; Ullmann et al., 2007), it is not yet clear to what extent the new drugs will affect the overall incidence and mortality caused by fungal disease. This is the result of their limited antifungal spectrum, the emergence of new, poorly susceptible filamentous fungi, and the difficulties encountered in rapid and accurate diagnosis of invasive infection. Furthermore, drug interactions and environmental moulds continue to be challenging aspects of disease control (Maertens, 2007; Bodey, 2005). 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 despite the introduction of the new agents, which are almost all effective against Candida sp (Pfaller et al., 2007). In experimental animal models of candidiasis, optimal antifungal protection has been achieved by vaccination with an attenuated low-virulence strain or after spontaneous recovery from initial infection (Bistoni et al., 1986; Cassone et al., 1988; Fidel et al., 1998; Romani et al., 1992). Since candidiasis is especially prevalent among immunocompromised subjects, however, the use of inactivated whole-cell or subunit vaccines should be a safer and more convenient approach.

Immune Response Against Candida
The importance of antibody immunity against a pathogen is usually inferred from one or more of the following criteria:

♦ Correlation of the presence of specific antibody with protection against infection.
♦ Prevention or modification of infection by antibody administration.
♦ Association of susceptibility to infection with antibody deficiencies.

*In vitro* studies demonstrating antibody-mediated killing or enhancement of cellular activity provide supportive evidence for protective antibody immunity. The term ‘protective antibody’ is applied here to antibodies that either prevent infection or modify the cause of infection to the benefit of the host (Robbins *et al*., 1995). The protective role of innate immunity, such as mechanical barriers and phagocytes, 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 have also been shown to have a role (Ip *et al*., 2004; Lillegard *et al*., 2006). Recent studies have highlighted the crucial role of dendritic cells in linking the innate to adaptive immunity and organizing the nature and extent of antifungal defense (Shoham *et al*., 2005; Levitz, 2004; Bernardis *et al*., 2006). Cell mediated immunity is commonly believed to be the primary defense against fungal diseases (Cutler *et al*., 2007; Deepe *et al*., 2005; Morris *et al*., 2006).

Important points to consider in antifungal immunity and its relevance to vaccination are:

♦ usually fungi display only moderate virulence (Gow *et al*., 2002; Latge *et al*., 2002).
♦ antifungal immune responses are usually redundant.

Although almost all pathogenic fungi have mechanisms to evade or intoxicate immune responses residual immunity may still be beneficial to the host (Monari *et al*., 2006; Gartner *et al*., 2005; Wheeler *et al*., 2006). Finally there is no need for a vaccine to be fungus-eradicating: neutralization of adhesins and enzymes or other low-penetration virulence traits may be sufficient to avoid disease (Cassone *et al*., 2006).

**Mechanism of antibody-mediated protection**

Protective immune sera, mucosal antibodies, some murine and human monoclonal antibodies, and genetically engineered antibody fragments have all shown remarkable efficacy in fighting fungi (Cutler *et al*., 2007; Cassone *et al*., 2006; Casadevall *et al*., 2002). In principle, antibodies can be induced by vaccination in at risk patients before they become immunocompromised. Furthermore, Because of the longevity of Ig G (weeks to months depending on the Ig G subclass), antibodies might persist with a protective titre even during prolonged immunosuppression.

Antibodies to *Candida albicans* agglutinate yeast cells could theoretically contribute to host defense by localizing infection. However, an agglutinating nonprotective Ig M MAb to *Candida albicans* has been described suggesting that the ability to agglutinate yeast cells is not sufficient for protection (Han *et al*., 1995). Ig G to *Candida albicans* prevent serum-induced clumping, a phenomenon of
uncertain physiological significance (Chilgren et al., 1968). Interference of *Candida albicans* with
attachment is a potent mechanism of antibody protection (Cassone et al., 1995; Epstein et al., 1982,
Han et al., 1995; Scheld et al., 1983; Umazume et al., 1995; Vudhichamnong et al., 1982). For
*Candida albicans* there is minimal phagocytosis by host effector cells in the absence of either
antibody or complement-derived opsonins (Chilgren et al., 1968). Antibodies to *Candida albicans* are
potent opsonins; however, opsonic antibody is not an absolute requirement for phagocytosis because
the yeast can stimulate the complement pathway (Solomkin et al., 1978). Specific Ig G has no direct
effects on *Candida albicans* growth (Chilgren et al., 1968), but Fab fragments to a hyphal antigen can
inhibit germ tube formation (Casanova et al., 1990). Antibodies to *Candida albicans* can absorb
immunosuppressive polysaccharide antigen from sera, suggesting a role for antibody in neutralization
of immunomodulating fungal products (Fischer et al., 1978). Thus for *Candida albicans*, Antibody
immunity may contribute to host defense by direct candidacidal activity (Poloneilli et al., 1994),
Prevent attachment (Epstein et al., 1982; Han et al., 1999; Scheld et al., 1983; Umazume et al., 1995),
Providing opsonins for more efficient phagocytosis (Chilgren et al., 1978), Binding to
immunomodulating polysaccharides (Fischer et al., 1978), Neutralizing extracellular proteases
(Cassone et al., 1995), Inhibiting the yeast-to-mycelium transition (Casanova et al., 1990), which is
associated with increased adherence and invasion.

**Antibody mediated enhancement of fungal infection**

Some antibody responses to fungal antigens may be deleterious to the host. Rabbits treated with
immune sera had more severe lesions than controls (Hurd et al., 1953). *In vitro* observations suggest
mechanisms by which antibody could contribute to the pathogenesis in *Candida albicans* infections.
Sera from certain patients with Candidiasis with high titers of antibody to *Candida albicans* can
interfere with neutrophils candidacidal activity (LaForce et al., 1975; Walker et al., 1980). Non-
specific IgA can enhance *Candida albicans* adherence to epithelial cells (Vudhichamnong et al.,
1982). The phenomenon of antibody mediated inhibition of serum-induced clumping (Preilser et al.,
1969) may contribute to dissemination by promoting mycelial transformation (Louria et al., 1972).
Antibody to *Candida albicans* can inhibit human lymphocyte proliferative responses to *Candida
albicans* antigen, possibly by interfering with macrophage antigen presentation (Witkin, 1986). An Ig
G like molecule has been implicated in the chemotaxis defect of a patient with mucocutaneous
candidiasis (Cates et al., 1980).

**Important considerations in studies of antibody protection**

The evaluation of the role of antibody immunity in animal systems involves complex experiments in
which the outcome is dependent on multiple variables including antibody quantity, specificity, and
isotype composition, inoculum, the timing of infection and antibody administration; route of infection
and antibody administration, the virulence of the experimental strain, and the susceptibility of the animal host to infection with the organism (Table 1).

Specific vaccines and antibodies

Table 3 summarizes some of the anticandida vaccines that have successfully provided both active and passive immunization. Almost all types of chemical and antigenic formulations, including antigen-encoding DNA, have been considered for active vaccination. 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. Advances in whole genome sequencing and proteomics 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 (Giefing et al., 2007; Thomas et al., 2006; Rappuoli et al., 2004). Recent examples of the application of this “antigenome” approach (Giefing et al., 2007) have been provided by Thomas et al (2006) for anticandidal vaccine. Attenuated fungal cells are potently protective vaccines in animal models (eg, the CA2 strain of Candida albicans) (Romani, 2004; Bistoni et al., 1986) but could not be used in immunocompromised patients.

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 polysaccharidized render sufficiently immunogenic through conjugation with a protein carrier (mostly bacterial toxoids) (Torosantucci et al., 2005; Han et al., 1999; Oscarson et al., 2005). Polysaccharide subunit vaccines include those based on original approaches such as peptide mimotopes (Datta et al., 2006; Maitto et al., 2004) and yeast killer toxin-neutralising antibody (Polonelli et al., 1993; Cassone et al., 1997; Polonelli et al., 1994). Some subunit vaccines are based on antigens that are common in different fungal species (Ibrahim et al., 2001; Spellberg et al., 2006) or even genera (Torosantacci et al., 2005; Cassone et al., 2006), raising the possibility of immunization against several fungi with a single antigenic formulation (the so called universal antifungal vaccine) (Torosantacci ., 2005). 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 andidiasis.

Table 1: Variables, experimental considerations, and design of antibody protection experiments (Casadevall, 1995).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experimental considerations</th>
<th>Suggestions</th>
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<tbody>
<tr>
<td>Antibody preparation</td>
<td>Polyclonal preparations are complex mixtures which may contain protective, nonprotective, and deleterious antibodies; the amount of specific antibody in polyclonal preparations may be</td>
<td>Use MAbs to defined antigens; if MAbs fail to modify infection, consider isotype switching since antibody efficacy may depend on constant-region functions; switching from IgG3 to IgG1</td>
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<tr>
<td>Parameter</td>
<td>Description</td>
<td>Notes</td>
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<tr>
<td><strong>Antibody dose</strong></td>
<td>Small doses may be insufficient for protection; very high doses may result in diminished antibody efficacy (i.e., prozone phenomena described with antipneumococcal antibodies (Felton, 1928)).</td>
<td>Titrate antibodies dose and inocula.</td>
</tr>
<tr>
<td><strong>Timing of antibody administration</strong></td>
<td>Antibody efficacy may depend on timing of antibody administration; antibody prophylaxis is usually more effective than therapy.</td>
<td>Administer antibodies before infection to maximize likelihood of demonstrating antibody protection</td>
</tr>
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<td><strong>Fungal strains</strong></td>
<td>Fungal strains can vary in susceptibility to antibody immunity (Mukherjee et al., 1995).</td>
<td>Test multiple strains of pathogen in question.</td>
</tr>
<tr>
<td><strong>Inoculum</strong></td>
<td>Small inocula may not produce reliable infections; large inocula may result in overwhelming infection refractory to antibody administration.</td>
<td>Use smallest inocula required to infect the majority of animals and produce the intended outcome (i.e., death, tissue infection etc.)</td>
</tr>
<tr>
<td><strong>Experimental animal</strong></td>
<td>Demonstration of antibody efficacy may be easier in some animal species; a GXM MAb prolonged survival in complement-deficient DBA/2J but not BALB/c mice (Dromer et al., 1989).</td>
<td>Consider testing antibody reagents in various animal models</td>
</tr>
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<td><strong>Route of infection</strong></td>
<td>Antibody efficacy may depend on the route of infection; for example, antibody efficacy against some pneumococcal strains was greater in i.v. infection than in i.p. infection (Briles et al., 1992).</td>
<td>Consider various routes of infection in experimental design; for example, rabbit polyclonal immune sera against C. neoformans prolonged survival in i.p. infection but not i.v. infection (Graybill et al., 1981).</td>
</tr>
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<td><strong>Parameters of antibody efficacy</strong></td>
<td>Survival, CFU, and severity of pathological lesions are frequently used parameters of antibody efficacy; organ CFU may be a more sensitive parameter of antibody efficacy than survival (Mukherjee et al., 1995)</td>
<td>Test multiple parameters; antibodies to C. neoformans can reduce tissue CFU without prolonging survival (Mukherjee et al., 1995; Sanford et al., 1990)</td>
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</table>
Table 2: The biological and pathological features of *Candida* spp. currently considered as important target for vaccines.

<table>
<thead>
<tr>
<th>Candida spp.</th>
<th>Biology</th>
<th>Pathogenecity</th>
<th>Disease</th>
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<tbody>
<tr>
<td>Several species of which <em>Candida albicans</em> is the most pathogenic. <em>C. albicans</em> can grow as both yeast and mycelial forms (hyphae), which are prevalent at 37°C. Pseudo-hyphae can also be formed. <em>C. albicans</em> are commensal organisms of the human gastrointestinal tract with a worldwide distribution.</td>
<td>Extracellular pathogens possess well defined virulence traits such as various adhesins and aspartic proteinase enzymes. Hyphae formation also contributes to virulence in vivo.</td>
<td>Cause superficial infections (skin and various mucosae, particularly vaginal and oral) and deep seated infections, in nearly all internal organs. Vaginal infection with <em>Candida</em> spp is estrogen dependent, and probably the most diffuse fungal infection worldwide, affecting around 75% of all women in fertile age at least once.</td>
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</table>

Table 3: Major fungal vaccines for active and passive immunization against candididiasis (Cassone, 2008).

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Underlying immunity</th>
<th>References</th>
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<tr>
<td>Whole cells and cell extracts</td>
<td>Strain CA2, live-attenuated</td>
<td>(Bozza et al., 2004; Bistoni et al., 1986)</td>
</tr>
<tr>
<td>Ribosomal cell fraction</td>
<td>T-helper 1, cell-mediated immunity</td>
<td>Segal et al., 2006; Levy et al., 1989</td>
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<tr>
<td>Inactivated whole cells</td>
<td>Antibodies and cell mediated immunity</td>
<td>Cardenas et al., 1999</td>
</tr>
<tr>
<td>Antigen pulsed cells and T cells</td>
<td>Dendritic cell loaded with Candida antigen</td>
<td>Bozza et al., 2004; Perrucio et al., 2004; Bacci et al., 2002</td>
</tr>
<tr>
<td>Subunit and glycoconjugates</td>
<td>Agglutin like sequences</td>
<td>Cutler et al., 2007; Ibrahim et al., 2006; Spellberg et al., 2006</td>
</tr>
<tr>
<td>Secreted aspartic proteases 2</td>
<td>Cell mediated immunity</td>
<td>Cassone et al., 1995</td>
</tr>
<tr>
<td>65Kda mannoprotein</td>
<td>Anti sap2 antibodies</td>
<td>Sandini et al., 2007</td>
</tr>
<tr>
<td>β-1,3-glucan</td>
<td>Adhesin neutralizing antibodies</td>
<td>Torosantucci et al., 2005; Cassone et al., 2006</td>
</tr>
<tr>
<td>β-1,2-mannosides</td>
<td>Growth inhibitory and cytocidal antibodies</td>
<td>Cutler et al., 2007; Cutler et al., 2005; Han et al., 1999</td>
</tr>
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</table>
Clinical trials of active and passive vaccination

There is no fungal vaccine approved or currently undergoing advanced clinical trials for active immunization in human beings. However, several vaccine manufacturers have fungal antigens under development as candidate vaccines. Vaccine formulation that have undergone limited phase I and phase II trials is against vulvovaginal candidiasis by a candida ribosome preparation (Levy et al., 1989). The result of this trial 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.

References


