REVIEW

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Vaccines against *Coccidioides*

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Vaccines against fungal diseases are gaining attention because of their growing impact on modern medicine. Development of these vaccines should incorporate immunological tools that integrate with or replace chemotherapy to minimize antibiotic use and consequent resistance. In this review, we evaluate the current developmental status of fungal vaccines against coccidioidomycosis. There is a need for a vaccine that sufficiently prevents disease, without eradicating the fungus, by neutralizing adhesions and enzymes or other low penetrance virulence traits.

Keywords: Fungal vaccines; Coccidioidomycosis

INTRODUCTION

Prevention of an infectious disease is preferable to treatment of the disease. Successful vaccines eradicating polio and smallpox have saved countless lives and healthcare dollars over the last 60 years. Furthermore, prevention of childhood diseases such as rubella, mumps, and pertussis, as well as the more recent vaccines protecting against some pneumococcal infections, has been extremely beneficial [1]. Vaccines targeting fungal diseases are gaining attention because of the growing impact of fungal diseases in modern medicine and the need to invest in immunological tools to integrate with or replace chemotherapy, which will minimize antibiotic use and consequent resistance. To date, only two commercial fungal vaccines have been developed; Russian and Czech veterinary vaccines against ringworm [2].

The least immunocompromised patient groups should be vaccinated against fungal diseases. These groups include chronic granulomatous disease patients, transplant candidates prior to transplant, leukemia patients after successful induction therapy, solid tumor patients at diagnosis, patients with rheumatic or inflammatory bowel disease before immunosuppression, and intensive care unit patients in high risk groups for aspergillosis who lack the classic risk factors, such as steroid use and cytotoxic chemotherapy [2]. A vaccine would be useful if it augmented an impaired immune response, 'held the fort' until immunity recovered, or acted synergistically with antifungal therapy [2]. Pioneer studies showed that cell-mediated immunity is stimulated by a fungal vaccine [2]. However, antibodies can be a correlate of protection against infection through activation of complement with neutralization of virulence traits, and opsonization, which affects the direction and vigor of cell-mediated immunity to inhibit fungal growth, adherence and germina-

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tion. Antibody affinity, specificity, and time of appearance or administration are the key components of antibody efficacy [2].

COCCIDIOIDOMYCOSIS

Coccidioidomycosis, also known as Valley fever, is a fungal disease caused by the closely related fungal species *Coccidioides immitis* and *Coccidioides posadasii*. The disease is endemic to the southwestern United States and areas of Latin America. The pathogen resides in soil, and arthroconidia arising from hyphal growth may become airborne if the soil is disturbed, thereby infecting humans and animals via the respiratory tract. After inhalation, the arthroconidia transform into endosporulating spherules in infected tissues. There are two clinical forms of coccidioidomycosis. One is a mild form that causes an influenza-like illness, which is a completely unapparent or can progress to a moderately severe disease, followed by complete resolution of the infection and establishment of strong immunity to reinfection. The second is a rare form in which the infection becomes established and is followed by a chronic progressive disease or an acute, rapidly fatal dissemination to the meninges, bones, joints, and subcutaneous and cutaneous tissues. Such involvement is characterized by the formation of burrowing abscesses progressing to a granulomatous reaction [1].

Recovery from coccidioidomycosis results in an apparent lifelong immunity to the disease. A vaccine is needed to induce a type 1 helper T cell (Th1) response against coccidioidomycosis that develops into protective host resistance. Certain population groups, such as Filipinos, appear to be at particularly high risk of developing coccidioidomycosis [3]. The annual incidence of coccidioidomycosis in the United States is 100,000 to 150,000 infections per year, of which 60% are asymptomatic and 40% are symptomatic infections [1]. The market for a vaccine to prevent Valley fever could be as large as 20 million residents and visitors to the endemic southwest [1].

T cell-mediated immunity is the most critical arm of the immune response to *Coccidioides* [1]. Polymorphonuclear leukocytes in the innate immune response are able to kill the organism [1], and are slightly more effective in inhibiting the growth of arthroconidia than that of mature spherules [1]. Pretreatment of macrophages with interferon (IFN)-γ and/or tumor necrosis factor-α can enhance the host's ability to kill arthroconidia *in vitro* [1]. A Th1 response is considered protective and a Th2 response nonprotective [1]. *Coccidioides* antigens also induce antibody responses during infection, which has very little benefit since high antibody titers typically correlate with a poor clinical outcome [1]. However, recent evidence suggests that humoral immunity is important in contributing to host resistance against fungi [1]. Particular antibody subsets act as opsonins against arthroconidia and endospores [1]. Thus, inhaled arthroconidia reach the alveolus, and interact with pulmonary dendritic cells (DCs) that process antigen; the DCs migrate to local lymph nodes where they present antigen and activate lymphocytes; the lymphocytes migrate back to the focus of pulmonary infection where they secrete inflammatory cytokines and initiate a granulomatous response. When this response is insufficient, the arthroconidia undergo morphological changes to endospores and spherules, and these latter forms may gain access to the blood stream, leading to dissemination. At dissemination sites, the proinflammatory response may be repeated and local antigen-processing cells recruited to augment the response [1]. As antigen-presenting cells (APC), DC process the antigen and present its epitopes to T cells within the context of major histocompatibility (MHC) I or II molecules. Pattern recognition receptors (PRRs) on DCs interact with surface-exposed, highly conserved molecules known as pathogen-associated molecular patterns (PAMPs), such as mannoproteins and β-glucan in fungi, and transduce signals for early inflammatory and nonspecific responses. PRRs include Toll-like receptors (TLRs), complement receptor 3, mannose receptor, Fcrγ receptor, and Dectin-1 [3]. TLR2 and TLR4 are involved in antifungal responses. PAMP-PRR interaction triggers a complex cascade of intracellular signaling that ultimately leads to the production of cytokines such as interleukin (IL)-12 and IL-23, activation and differentiation of naïve T cells into antigen-specific $CD4^+$ Th or $CD8^+$ T cells, and expression of antifungal activity by the humoral and cellular arms of adaptive immunity [3].

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ANTIBODIES

Protective immune sera, mucosal antibodies, murine and human monoclonal antibodies, and genetically engineered antibody fragments have all shown remarkable efficacy against fungi [3]. Mycograb is a monoclonal recombinant antibody directed against heat shock protein. A randomized, blinded, multicenter trial compared treatment of invasive candidiasis with liposomal amphotericin B only or in combination with Mycograb in 117 patients. The results demonstrated that combined treatment was superior to chemotherapy alone. The antibody was well tolerated, with the possible exception of hypertension in some patients following the initial dose [4].

ADJUVANTS

The importance of adjuvants in enhancing and directing the immune response to vaccines is critically important. In general, adjuvants that delay the release of soluble antigen over time can enhance the response by extending exposure of immune cells to antigen [1]. Soluble antigens can be more effective by being incorporated into a carrier system that distributes the antigen more efficiently to cells of the immune system (e.g., lipid complexes), thereby facilitating uptake by APCs or by attracting more APCs to the site of antigen localization [1]. Alum-based vaccines shift the immune response toward humoral immunity, rather than cellular immunity [1]. However, these adjuvants have side effects when administered by parenteral routes. CpG adjuvants are effective in inducing a Th1 response, but cause adverse side effects upon repeated administration [1]. Liposomes have previously been successfully explored as a delivery system for a fungal vaccine against *Candida albicans* [1]. An exciting development would be adjuvants that are efficacious for antigen presentation by the oral route. This would greatly simplify administration, reduce side effects, and allow for rapid vaccination of large numbers of people. However, an oral vaccine may not be feasible since orally administered antigens result in development of tolerance. One potential method for oral administration is incorporation of specific antigens into liposomes, which prevent degradation of antigen in the gut and induce a systemic immune response [1].

VACCINES AGAINST *COCCIDIOIDES*

Several vaccine preparations have been developed against *Coccidioides*. The first type of preparation uses killed vaccines. The formalin-killed spherule (FKS) vaccine provided significant protection in animal models, but has failed to demonstrate a significant reduction in incidence or severity of disease in phase III trials because of inadequate dosing of the FKS due to associated side-effects such as local irritating pain. A possible direction for future research is to attempt to remove these irritants while preserving the key immunogens by fractionating the vaccine components. The second type of preparation uses auxotrophic mutants or isolates with reduced virulence. For example, gene-deletion mutants of a chitin synthase gene CHS5, or double mutants disrupting both CTS2 and CTS3 have been generated as avirulent types unable to endosporulate, and have shown good protection against experimental pulmonary infection. These vaccines provide a survival advantage, but do not result in complete clearance of *Coccidioides* from tissue. Live-cell vaccines are an unlikely third candidate because of possible reversion to virulence of an attenuated mutant. The fourth type of preparation consists of protein and subcellular vaccines. A recent focus of *Coccidioides* vaccine efforts has been on more purified antigens, including those produced by recombinant DNA technology. These include urease (Ure), gel1, Pmp1, antigen 2/proline-rich antigen (Ag2/PRA), SOW, ELI1, *Coccidioides*-specific antigen (CSA), aspartyl protease, and heat shock protein 60. Protein vaccine candidates, including PRA, chimeric protein consisting of PRA and CSA, multivalent vaccine consisting of Pep1, P1b, and Amn1, have also been proposed. DNA vaccines induce superior protection and this may provide a future direction for additional research. The fifth type of preparation is a DC vaccine. DCs were transformed with a plasmid encoding the AG2/PRA protein and instilled intranasally into mice prior to infection. This vaccine significantly reduced fungal burden, induced higher levels of IFN-γ, and caused less severe pathology in mice. The sixth candi-

date is protein-glycan conjugate vaccines. Glucan, a pleiotropic immunomodulator binding to the dectin-1 receptor, has been shown to stimulate immunity to bacteria, viruses, parasites, and fungi. This type of vaccine increased phagocytosis and antimicrobial killing, stimulated nitric oxide production, activated complement, primed spleen cells for cytokine production, stimulated IL-17 production, increased natural killer cell activity, increased costimulatory molecules, activated DCs, and stimulated DC maturation, increased matrix metalloproteinases, stimulated hematopoiesis, mobilized stem cells to the periphery, and was anti-inflammatory. Mannans deliver antigens to the MHC, enhance maturation of DCs, increase surface expression of CD40 and other markers, and increase production of many cytokines. Oral glucan can be an immunostimulant, and stimulate resistance to infection [1,2]. *Aspergillus* and *Candida* appear to share glucan epitopes and glucans act as adjuvants with fungal antigens [1]. Recent studies suggest the importance of glycans in stimulating a protective antifungal response and that could lead to possible pan-fungal immunoprotection. Mannan is a pleiotropic immunomodulator, binding to the mannose receptor, TLRs, and others [2]. The structure and composition of mannan is species-specific [2]. The key mannan in *Aspergillus* is galactomannan [2].

A mannose trisaccharide glycan-protein from the *Candida* cell wall yielded an antibody response to both components, and provided a protective response against candidiasis, though the choice of protein was critical for elicitation of a protective response [2]. The most productive route to a fungal-specific vaccine may be to work towards a conjugate vaccine that combines the optimally configured glycan with a specific immunogenic protein. Some proteins may be sufficiently cross-immunogenic, such that combined with the appropriate glycan, it may be possible to develop a pan-fungal vaccine. A preliminary study investigated the potential of glucan or mannan alone or conjugated to bovine serum albumin as protective vaccines against *Coccidioides* and *Aspergillus*, and showed promise as potential vaccine candidates. When conjugating protein to glycans, how the protein and carbohydrate are coupled is a key step. The carbohydrate can be destroyed, there can be undesirable intrachain and interchain crosslinking of the polysaccharide, or several polysaccharide chains can attach to a protein molecule at multiple sites, resulting in high molecular weight aggregates, which is undesirable [2]. Coupling methods that have been successful include triethylene glycol acrylate and 1-cyano-4-dimethylaminopyridium tetrafluoroborate (CDAP) [2]. CDAP is less hazardous, easier to use, and more rapidly active.

The use of heat to kill the yeast increases the binding of β-glucan by dectin-1, at least in part by increasing exposure of binding sites, and further, heat denaturation of proteins tends to shift the host response against them to a Th1 type [2]. Examples of cross-reactive proteins include AspF3 from *Aspergillus fumigatus*, Pmp1 from *Coccidioides*, and Ahp1 from *Saccharomyces cerevisiae*. Others include chitinase cts2 in *Coccidioides*, cts1 chitinase in *Saccharomyces*, and chitinases of *Aspergillus*; cell wall proteins Gel1p in *Aspergillus* and *Coccidioides*, with Gas1p in *Sacharomyces*; and Crh1 in *Saccharomyces* and Crf1p in *Aspergillus* [2]. Seven of the 20 most abundant proteins of *A. fumigatus* and *C. posadasii* hyphae share >50% sequence identity [2].

UNIVERSAL VACCINES

Several approaches to a universal vaccine against opportunistic fungal pathogens are possible. One is the use of laminarin, a β-glucan from algae, which was conjugated with a genetically detoxified diphtheria toxin and used to immunize and protect against both *Candida* and *Aspergillus* fungi [5]. The anti-β-glucan antibodies generated in the above vaccination and monoclonal antibodies sharing specificity proved also to be protective against *Cryptococcus* [6]. Importantly, all of these antibodies showed direct inhibition of these three pathogens in the absence of host cells. Directly inhibitory antibodies are uncommon and may have advantages for use in immunocompromised patients. The other approach is idiotypic vaccination [7], which is based on immunization with an antibody directed against a wide-spectrum yeast killer toxin. This induces anti-idiotypic antibodies that mimic the fungicidal activity of the killer toxin itself. Heat-killed yeast, which is a protein-carbohydrate conjugate vaccine, showed protection against aspergillosis, coccidioidomycosis, *C.*

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albicans, and *Cryptococcus neoformans* [1]. The mechanism is that yeast stimulated innate CD8 reactions, and uptake of yeast by DCs leads to increased expression of CD40, IL-12, costimulatory molecules, and increased MHC I- and II-restricted T cell responses [2]. The vaccine using the *Candida* adhesion Als3 is protective against both *C. albicans* and *Staphylococcus aureus*, two top-ranking causes of health care-associated infections [8]. β-Glucan-based fungal vaccines can generate fungicidal or fungus-growth-inhibitory antibodies [5]. Theoretically, bactericidal antibodies could be raised by immunization with functionally similar, highly conserved PAMP including peptidoglycan fragments, alone or conjugated to a carrier. These antibodies would act as antibiotics, and have been coined "antibiobodies" by Polonelli et al. [9]. These antibodies could be a breakthrough in the therapy of immunocompromised patients.

Seeveral potential limitations to universal vaccines exist. First, defocusing of the immune responses and then a decrease in the capacity to eliminate or keep at bay the etiologic agent. It may not be immunodominant, raising the issue of how to potentiate the dominance of antigenic determinants without excessive inflammation. The use of potent viral vectors, presentation as virus-like particles, conjugation with highly immunogenic carriers, and formulation with improved adjuvants such as oil-in-water mixtures or PAMP are some of the tools being exploited. All of the above, in particular the use of PAMP either as an antigen or as a carrier, conveys the possibility of raising autoimmune responses through molecular mimicry or even raising immune responses which dampen the capacity of the host to recognize molecular pattern signatures for a first-line antimicrobial defense. Finally, these broadly specific immune responses could significantly affect the human microbiota, causing excessive elimination of commensal microorganisms. Thus, careful dissection of host beneficial immunity from harmful responses is necessary [10].

CONCLUSIONS

There is a need for a vaccine that sufficiently prevents disease, without eradicating the fungus, by neutralizing adhesions and enzymes or other low penetrance virulence traits. Immunopreventive or immunotherapeutic interventions are novel approaches to combating fungal infections that deserve increased attention in the future.

Conflict of interest

No potential conflict of interest relevant to this article is reported.

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