Exploiting fungal cell wall components in vaccines

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Abstract Innate recognition of fungi leads to strong adaptive immunity. Investigators are trying to exploit this observation in vaccine development by combining antigens with evolutionarily conserved fungal cell wall carbohydrates to induce protective responses. Best studied is β-1,3-glucan, a glycan that activates complement and is recognized by dectin-1. Administration of antigens in association with β-1,3-glucan, either by direct conjugation or complexed in glucan particles, results in robust humoral and cellular immune responses. While the host has a host of mannose receptors, responses to fungal mannoproteins generally are amplified if cells are cooperatively stimulated with an additional danger signal such as a toll-like receptor agonist. Chitosan, a polycationic homopolymer of glucosamine manufactured by the deacetylation of chitin, is being studied as an adjuvant in DNA and protein-based vaccines. It appears particularly promising in mucosal vaccines. Finally, universal and organism-specific fungal vaccines have been formulated by conjugating fungal cell wall glycans to carrier proteins. A major challenge will be to advance these experimental findings so that at risk patients can be protected.

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Introduction

Vaccines are one of the greatest triumphs in the history of medicine [1]. Despite these gains, effective vaccines remain elusive for many infectious diseases. Moreover, many existing vaccines use live, attenuated organisms. While many live vaccines confer long-lasting immunity, they also tend to have a higher frequency of adverse events, including the potential to cause the infectious disease they are designed to protect against [2]. This is especially a problem for the ever growing population of immunocompromised individuals, in whom live vaccines are contraindicated because of the risk of disseminated infections.

Vaccines that contain killed whole organisms or purified antigens ("subunit vaccines") are intrinsically safer. However, purified antigens tend to not elicit robust immune responses unless administered with an adjuvant to augment the adaptive immune response and generate immunological memory [3]. One promising approach to improving subunit vaccines is to use vaccine platforms that function as delivery systems to efficiently target antigen to dendritic cells (DCs), the professional antigen-presenting cells of the immune system most responsible for initiating immune responses [1]. Critical then for vaccine design is the delivery system and the choice of adjuvants. The adjuvant used in most licensed subunit vaccines, alum, generally biases toward Th2-type antibody responses [1, 3, 4]. While antibody elicited by alum-adjuvanted vaccines is adequate to protect against many diseases, T cell defenses appear to be required to protect against many of the pathogens for which effective vaccines are lacking. In addition, vaccine-mediated T cell protection may work best if the response is skewed, as for example toward a Th1- or Th17-type response.

Innate defenses against fungi are robust and evolutionarily conserved in the animal kingdom. In mammals, if innate defenses fail, adaptive immunity gets triggered. The ongoing

"epidemic" of fungal infections is primarily driven by the increased numbers of persons with defects in one or more host defense mechanisms [5]. For example, the vast majority of the over one million estimated annual cases of cryptococcosis have T cell immunocompromise due to AIDS [6] with a lesser contribution from the use of immunosuppressive medications to treat autoimmune diseases and to prevent transplant rejection. The lesson is that when host defenses are intact, fungal infections are very rare. The question then arises whether this observation can be exploited in vaccine development. In other words, if innate recognition of fungi leads to strong adaptive immunity, can vaccines combine antigens with specific components of fungi to induce protective responses?

In this article, we review the studies examining this concept. Our focus is on the conserved carbohydrate components of the fungal cell wall that are recognized by receptors on dendritic cells (Fig. 1). These glycans include β-glucans (β-1,3-linked polymers of glucose with β-1,6 branches), chitin (homopolymer of *N*-acetylglucosamine); chitosan (deacetylated chitin), and mannans (mannose chains of varying lengths and configurations added to fungal proteins via N- or O-linkages) [7]. Structurally, chitin and chitosan tends to be in the inner portion of the cell wall, β-glucans on the middle, and mannans on the outside. While the extent varies depending on the fungal species, mannans "mask" β-glucans, chitin, and chitosan, thereby impairing fungal recognition. Moreover, some fungi also have other masking glycans, including α-glucans and glucuronoxylomannan [8, 9].

β-Glucans

Even primitive invertebrates such as the horseshoe crab are able to sense β-glucans and mount an innate immune response [7]. In mammals, the major receptors that recognize β-glucans are dectin-1 and complement receptor 3 (CR3). The β-glucan recognition site on CR3 is distinct from the complement recognition site. The scavenger receptors CD5, CD36, and SCARF1 have also been reported to recognize β-glucans. Both β-1,3- and β-1,6-linked glucans potently activate the alternative pathway of complement resulting in fungal opsonization and the generation of chemotaxins which can recruit inflammatory cells [10].

The immunostimulatory properties of β-glucans were recognized long before the discovery of dectin-1 and other receptors for β-glucans. Notably, β-glucan preparations derived from fungi have a record of safety in both preclinical and human trials [11–13]. Stimulation via dectin-1 primes Th17, Th1, and cytotoxic T cell responses [14–16]. Injection of antigen targeted to dectin-1 induced $CD4^+$ and $CD8^+$ T cell responses at doses that antigen alone failed to produce a response [17]. Thus, a natural extension of these findings
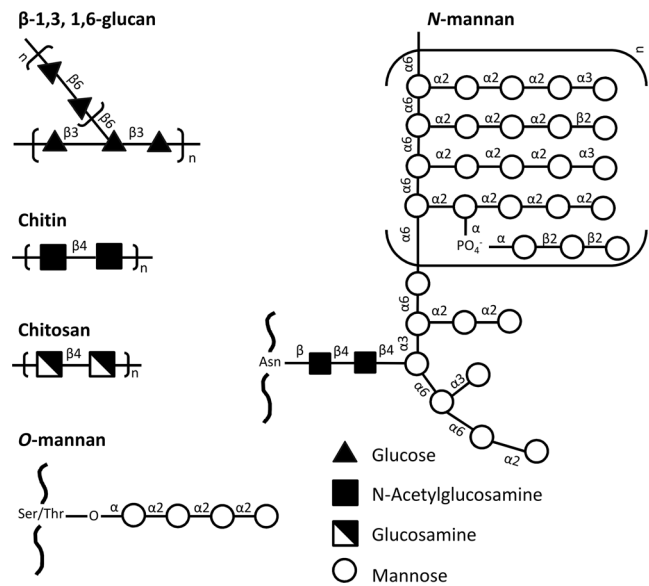

Fig. 1 Representative carbohydrate components of the fungal cell wall that have been exploited in vaccine design. Note that there is considerable interspecies and intraspecies variability, particularly in the structures of O- and N-mannans

has been to explore the use of β-glucans as vaccine adjuvants and delivery systems.

Our laboratories have extensively explored the use of glucan particles (GPs) as a vaccine delivery system. GPs are highly purified, hollow, porous cell wall shells derived from baker's yeast following a series of hot alkali and organic extractions. GPs are composed primarily of β-1,3-glucan although do contain some chitin and β-1,6-glucans. Using KO mice and receptor antagonists, we demonstrated that GPs are recognized by dectin-1 [18], a C-type lectin receptor highly expressed on phagocytes including myeloid dendritic cells (mDCs) [19]. If a source of complement is provided, GPs will activate complement and in vivo phagocytosis of GPs is mediated by complement receptors and dectin-1 [10, 20]. GPs stimulate mDCs to produce cytokines associated with beneficial responses in vaccine models [18]. Moreover, synergistic DC cytokine responses are seen when GPs are combined with toll-like receptor (TLR) agonists [18].

Polymer-complexed cores can be constructed within the hollow GPs, enabling the delivery to DCs and macrophages of a wide variety of "payload" classes including proteins, siRNA, DNA, and other small molecules [20–25] (Fig. 2). As a proof of principle, we have constructed GPs containing complexed ovalbumin (GP-OVA), exploiting the plethora of immunological tools available to study this model antigen. Robust and long-lasting antigen-specific humoral (IgG_1 and IgG_{2c}) and T cell (Th1- and Th17-biased) responses were observed following subcutaneous immunization of mice with GPs "encapsulated" with antigen [20, 24]. In contrast, consistent with alum being a poor stimulator of T cell responses,
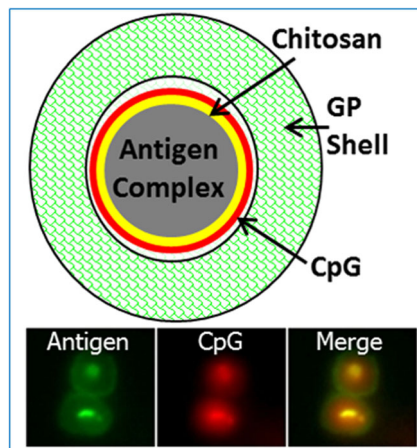
Fig. 2 Glucan particles (GPs). *Top*: Schematic showing the "layer-by-layer" design of a GP containing complexed antigen and CpG-rich DNA. *Bottom*: Epifluorescent photomicrographs of two abutting GPs loaded with antigen (fluorescently labeled *green* with FITC) and CpG (fluorescently labeled *red* with TRITC)

mice that received OVA admixed with alum predominantly exhibited IgG$_1$ antibody responses. Interestingly, the Th17 skewing after GP-based immunization was diminished by about 50 % in mice deficient in the third component of complement, but not in dectin-1$^{-/-}$ mice [20].

The above studies used yeast RNA to trap antigen within the GPs [20, 24]. As the long-term goal is to develop human vaccines, we have begun to formulate GP vaccines using pharmaceutical-grade materials generally regarded as safe. Mice were vaccinated with GPs containing OVA trapped with (1) yeast RNA, (2) alginate calcium, and (3) alginate calcium plus chitosan. Robust antigen-specific CD4$^+$ T cell and antibody responses were seen regardless of the trapping materials utilized [26]. Note that alginate calcium and chitosan are approved by the US Food and Drug Administration and generally regarded as safe [27, 28].

Ideal vaccines promote immune responses that are durable and of high quality [1]. To examine this, mice received 3 vaccinations of GP-OVA and were euthanized 20 months later. Single-cell suspensions of lymph node and spleens were then stimulated with OVA and analyzed by polychromatic flow cytometry for surface expression of CD3, CD4, and CD8 as well as intracellular staining for IFNγ, IL-17a, and IL-4. Unvaccinated mice served as controls. As expected, the naïve mice had virtually no CD4$^+$ T cells that stained for intracellular cytokines. However, 9.16, 2.16, and 0.31 % of the CD4$^+$ cells from the vaccinated mice were positive for intracellular IFNγ, IL-17a, and both cytokines, respectively [26]. Staining for IL-4 was not observed. In addition to durability of response, antigen sparing is desirable in vaccines [1]. We found that mice immunized with submicrogram doses of antigen still had brisk Th1- and Th17-biased CD4$^+$ T cell and antibody

responses [26]. Finally, we demonstrated that GPs function as both a delivery system and an adjuvant; antibody and T cell responses are seen when GPs and OVA are admixed but are substantially greater when OVA is encapsulated within the GPs [26]. In an unpublished work, we have demonstrated that vaccination of mice with GPs loaded with pathogen-specific antigens provides protection against certain lethal infections and validated the utility of GPs as a tool in vaccine antigen discovery.

Heat-killed *Saccharomyces cerevisiae* genetically engineered to express antigens are undergoing clinical trials as immunotherapeutic vaccines for patients with certain cancers and chronic infections [29, 30]. Importantly, in the human and animal studies, strong Ag-specific helper and cytotoxic T lymphocyte responses were elicited [29, 31–33]. The latter is especially notable because it has been generally difficult to elicit CD8$^+$ T cell responses using killed vaccines. The reported safety profile has thus far been favorable in phase I/II dose-escalation trials [29]. However, because yeast proteins, lipids, and nucleic acids are not eliminated, concerns regarding reactogenicity and autoimmunity could limit the appeal of this platform as a preventive vaccine given to predominantly healthy people.

Mannans

Mammalian and fungal cells share fundamental eukaryotic features of protein glycosylation; initial glycosylation occurs in the endoplasmic reticulum with further processing in the Golgi [34, 35]. However, whereas fully processed mammalian glycoproteins rarely have terminal mannose groups, N-linked and O-linked glycans on yeasts generally are terminally mannosylated. N-linked glycans on yeast proteins can be hundreds of sugars long and have extensive branching, while O-linked glycans tend to be linear chains of two to six mannoses [34–37]. Patterns of mannosylation can vary extensively when comparing different species of fungi. Strains and morphotypes within a species may also have heterogeneity of mannosylation.

Misfolded or incompletely processed mammalian proteins can have terminal mannosylation. Thus, a dilemma the host immune system faces is how to distinguish mannosylated self-proteins from foreign antigens. There is evidence that this is accomplished in two ways. First, responses are greatly amplified if cells are cooperatively stimulated with mannosylated ligands and toll-like receptor agonists [38]. Presumably, having the second "danger" signal tells the host it is likely dealing with a pathogen. Indeed, some studies have demonstrated immunological non-responsiveness against processed mannosylated antigens [39, 40]. Second, the host has multiple mannose receptors, with varying specificities with regard to

the molecular configurations of the mannose chains that are recognized and the signaling pathways that are triggered. Myeloid C-type lectin receptors with reported affinity for mannose that have been implicated in recognition of fungi include the mannose receptor (CD206), dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN; CD209), dectin-2 (CLEC6A), mincle (CLEC4E), and langerin (CD207) [41, 42].

C-type lectin receptors have cytoplasmic tails containing sorting motifs directing internalization into clathrin-coated vesicles. For example, mannosylated antigens taken up by the mannose receptor are endocytosed and released into the acidic environment of early endosomes. The mannose receptor then recycles to the cell surface while the released antigen is processed for subsequent presentation on MHC class II molecules [43]. This results in considerably more efficient intracellular degradation and antigen loading compared with macropinocytosis [44]. DC-SIGN also contains internalization motifs which target antigen for presentation to T cells [45]. Not surprisingly then, fungal mannosylation of antigens has been explored as a vaccination strategy.

For the encapsulated fungus, Cryptococcus neoformans, considerable evidence has accumulated that protein mannosylation drives T cell responses. Pioneering studies examined a crude culture supernatant which stimulated T cell responses in immunized mice. It was found that the mannoprotein fraction, defined by the ability to adhere to a concanavalin A affinity column, was predominantly responsible for these responses [46]. Subsequent work demonstrated that mannoproteins stimulate lymphoproliferation of CD4+ T cells obtained from HIV− to HIV+ cryptococcosis patients [47–50]. Moreover, most cryptococcal proteins identified on the basis of immunoreactivity have been found to be mannoproteins. For example, our laboratory made two T cell hybridomas from mice immunized with disrupted C. neoformans cells. The antigens that stimulated the two hybridomas were both mannoproteins (designated MP98 and MP88) that shared structural features including a signal sequence, a functional domain, a serine/threonine-rich region (which features extensive O-mannosylation), and an omega site for attachment of a glycosylphosphatidylinositol (GPI) anchor [35, 51, 52]. Moreover, we identified 53 other predicted mannoproteins that share these common features by in silico analysis [35].

To investigate the molecular basis of mannosylation in immune responses to cryptococcal proteins, MP98 was expressed recombinantly in a bacterium (Escherichia coli) and a yeast (Pichia pastoris), resulting in unglycosylated and mannosylated proteins, respectively [53]. In addition, P. pastoris MP98 was chemically deglycosylated. The Pichia-derived antigen stimulated significantly more antigen-specific cytokine production compared with the E. coli-derived antigen. Moreover, responses were potently inhibited if the antigen was chemically deglycosylated or if MR were blocked with mannans. A similar strategy was utilized with the model antigen, OVA [54]. Recombinant proteins containing immunoreactive portions of OVA were produced in P. pastoris with variations in the degree and type of glycosylation. The presence or absence of N-linked mannosylation was controlled by site-directed mutagenesis of potential N-linked glycosylation sites. Extensive O-linked mannosylation was accomplished by adding the S/T region of the cryptococcal mannoprotein, MP98. Completely unglycosylated counterparts were produced in E. coli. OVA containing N-linkages, extensive O-linkages, or both were more effective than unglycosylated Ags at inducing antigen-specific proliferation of CD4+ and CD8+ T cells in vitro [54–56]. This effect was apparently dependent on mannose receptors as mannan inhibited the enhanced response.

Others too have incorporated mannosylation of antigens or delivery systems as part of an experimental vaccination strategy [57]. While many of these systems effectively target antigen to the antigen-processing machinery of DCs, for reasons noted above, a second adjuvant may be required to elicit sustained immunity [58]. Finally, directing antigen to DCs via anti-DC-SIGN or anti-mannose receptor antibodies results in brisk antigen-specific T cell responses [59, 60].

Chitin and chitosan

Chitin, a homopolymer consisting of β-1,4-linked repeating units of N-acetylglucosamine, is second only to cellulose as the most common natural polysaccharide on our planet [61]. Deacetylation of chitin yields chitosan by the conversion of N-acetylglucosamine to glucosamine and the polymer from a neutral polysaccharide to one that is polycationic. Some fungi (e.g., species of Mucor and Cryptococcus) have, in addition to chitin, relatively large portions of chitosan as part of their vegetative cell wall or in the case of Saccharomyces, as part of the spore wall. In these organisms, chitin deacetylase(s) enzymatically remove the acetyl group from N-acetylglucosamine.

Chitin is an integral component of all fungal cell walls and exoskeletons of crustacea and insects, which collectively are the principal sources for environmental exposure to chitin [62]. Recent identification of chitin synthase genes in various other metazoans has reinforced the notion that chitin is an ancient polymer that has been integrated into a broad spectrum of eukaryotes. Thus, exposure to chitin is very common and can occur via a variety of routes including ingestion, inhalation, and cutaneous contact. Mammals lack chitin but express multiple enzymes capable of degrading chitin and chitosan; their microbiomes also have this capability.

In fungi, chitin is buried in the cell wall and covalently linked to β-glucans. Its exposure to the environment (or mammalian host) is shielded by glucans and mannoproteins. Chitin in the exoskeleton of insects and crustaceans is likewise sequestered, in complex with numerous chitin-binding proteins that organize chitin fibers into multiple layers. Insects also incorporate chitin into the peritrophic membrane that lines the gut. This chitin complexes with peritrophins and is periodically excreted. As chitin bearing organisms are degraded, chitin becomes more exposed.

Commercial preparations of chitin are typically made from crab and shrimp shell waste by a process that requires demineralization with acid and extraction with hot alkali. During this procedure, the chitin is partially deacetylated. Chitosan is made by extended treatment in hot alkali to remove additional acetyl groups. Both "chitin" and "chitosan" are generic terms used to reflect the degree of deacetylation of the polymer. Chitin has been ascribed to polymers with <40 % glucosamine and chitosan to ones having >60 % glucosamine. Native chitin and possibly chitosan however approach being the respective homopolymers [61].

Chitin and chitosan have very different physiochemical properties. Chitin polymers complex into higher-order crystalline-like structures and are insoluble in aqueous solutions; solubility is only gained upon hydrolysis to short oligosaccharides. Chitosan, regardless of polymer length, is soluble under mildly acidic conditions due to protonation of the amine group on glucosamine but aggregates into an insoluble form as the pH rises to neutrality. The primary amine of glucosamine has made chitosan very versatile in biomedical applications as it enhances interaction with negatively charged biomolecules and its ease of being derivatized.

There may be multiple mammalian receptors for chitin and chitosan. One receptor of chitin is FlCBD1, which is an endocytic membrane protein that binds the acetyl group of chitin through an extracellular, fibrinogen-like, recognition domain. It is expressed on enterocytes of the gut and on airway and salivary epithelial cells [63]. Other proteins that bind chitin are secreted. These include the mammalian chitinases and chitinase-like proteins, which interact through chitin-binding domains. Their importance is in degradation, as well as in regulating immune responses to chitin [64]. Another secreted protein RegIIIγ or HIP/PAP (its human counterpart) binds the peptidoglycan of gram-positive bacteria and is bactericidal. Chitin and the glycan portion of the peptidoglycan have similar structures [65]. As chitin becomes deacetylated and converts to chitosan, the polymer becomes a polycation and interacts with negatively charged biomolecules, such as mucin, hyaluronan, nucleic acids, and fatty acids. These charge-based interactions could complicate efforts to identify a specific receptor for chitosan.

A variety of immunological effects have been attributed to chitin and/or chitosan, including stimulation of proinflammatory cytokines, contribution to asthma, and protection against infections and cancers [64]. In recent studies, highly purified chitin too large to be phagocytosed was shown to induce in the lungs of mice the release of Th2 cytokines IL-5 and IL-13, which directly led to accumulation of eosinophils and alternatively activated macrophages [66]. Lung γδT cells were also activated to secrete IL-17 (Th-17 skewing) accompanied by increases in cytokines TNF-α, IL-1, and IL-23 (Th-1 skewing). Phagocytosis of pure chitin by mouse macrophages and dendritic cells, as well as human monocytes, had little effect on cytokine secretion [67]. However, phagocytosed chitosan activated the NLRP3 inflammasome with secretion of proinflammatory cytokines IL-1β and IL-18.

There is increasing use of chitin and chitosan in the biomedical industry. For example, chitosan nanofibrils of varying shapes have been studied as drug, nucleic acid, and protein delivery systems, as tissue engineering scaffolds to mimic an extracellular matrix, as promoters of wound healing, and as anti-microbials and vaccine adjuvants [68, 69]. The only FDA-approved use of chitosan, however, is in hemostatic bandages under emergency conditions.

Chitosan has been extensively studied as a vaccine adjuvant, mainly for use under oral and nasal administration. Mucosal vaccines are designed to target and augment immunity at areas of the gut and respiratory and vaginal tracts that would first encounter a pathogen. Their reported advantage over vaccines delivered to non-mucosal tissues is the induction of antigen-specific, secreted IgA by B cells underlying the mucosal epithelium [70]. FDA-approved vaccines against influenza by nasal delivery and viruses and bacteria by oral delivery have used live, attenuated strains (influenza, polio, rotavirus, *Salmonella typhi*, or heat-killed cells (*Vibrio cholerae*)) to induce immunity. The challenge has been to develop subunit vaccines that effectively deliver antigens across mucus-lined epithelium, bypassing hydrolytic and oxidative enzymes, to innate immune cells and lymphoid tissues. Chitosan has several properties that make it an attractive vaccine adjuvant [71]. As a polycation, it is mucoadhesive through interaction with negatively charged sialic acid of mucin. Together with its ability to open tight junctions between epithelial cells, antigen is more effectively delivered to antigen-presenting cells. Chitosan is non-toxic, is slowly degraded, and protects DNA plasmid- and protein-based vaccines from hydrolysis [28].

The effectiveness of chitosan as an adjuvant for intranasal delivery of influenza, pertussis, and diphtheria vaccines was demonstrated in animal studies with protection to challenge, underlined by superior IgA responses and equivalent IgG responses to vaccination by injection [72]. Studies continue

to demonstrate that chitosan is an effective adjuvant for intranasal vaccines that promotes a protective cross-protective immune response against various strains of influenza [73].

While not typically studied by injection because its mucoadhesive properties become muted, the high viscosity of chitosan aids in a prolonged release of antigen. In mice, antigen mixed with chitosan augmented both antibody and cell-mediated responses over alum as adjuvant [74]. When the amine groups on the polymer are partially derivatized by attachment of mannose, immune responses are superior to unmodified chitosan by intranasal vaccination. The addition of mannose improved targeting to antigen-presenting cells expressing the mannose receptor [75].

Carbohydrate conjugate and mimeotope vaccines

Millions of cases of life-threatening fungal infections occur annually, mostly in immunosuppressed persons [5]. Other mycoses, such as recurrent vulvovaginal candidiasis, can cause considerable morbidity in those afflicted. Thus, efforts have focused on developing vaccines that prevent development of fungal infections. Conserved cell wall glycans have appeal as "universal" fungal vaccines while glycans present on only some fungal species are candidate antigens for organism-specific vaccines. However, with rare exceptions, glycans do not elicit T cell responses and most fungal glycans are poor elicitors of antibody responses. To circumvent the latter problem, conjugate vaccines consisting of fungal glycans covalently linked to carrier proteins have been synthesized.

A "pan-fungal" conjugate vaccine consisting of laminarin (an algal derived β-1,3-D-glucan) linked to diphtheria toxoid (DT) was protective in models of candidiasis and aspergillosis and is in clinical trials [76, 77]. Protection appears to be due to the development of a protective anti-β-glucan antibody response. While laminarin is recognized by β-glucan receptors, it generally acts as an antagonist [78], so the role the contribution receptor targeting plays to the efficacy of this vaccine is not clear. Less work has been performed examining the potential role of β-1,6-D-glucans. Neutrophils preferentially recognize β-1,6-D-glucans over β-1,3-D-glucans [79], but it remains speculative whether directed targeting of neutrophils would be of benefit in vaccine design.

Among medically important fungi, the *C. neoformans* species complex is unique in that it contains a capsule of which glucuronoxylomannan (GXM) is the major capsular component. A conjugate vaccine consisting of GXM linked to tetanus toxoid was immunogenic and partially protective in mice [80, 81]. A peptide mimetic of GXM also induced protective responses against *C. neoformans* in mice [82]. In contrast, a conjugate vaccine consisting of another cryptococcal capsular component, galactoxylomannan, elicited non-opsonic Abs

and the vaccine was not protective [83]. Other work has focused on conjugating β-1,2-mannotriose, which is found in the cell wall of *Candida* species, to a combination of tetanus toxoid and surface proteins of *Candida albicans*. Remarkably, immunization with some of these chimeric vaccines completely protected mice lethally challenged with *C. albicans*, even in the absence of adjuvants [84]. An appeal of this system is it generates responses against both protein and carbohydrate components of the fungal cell wall.

Other promising vaccine approaches are focused on elicited antibody and/or T cell responses to fungal proteins [85–88] or employ live fungi with deletions of virulence factors [89]. For example, a live, attenuated strain of *Blastomyces dermatitidis* null for the adhesion BAD1 was found to protect mice from three endemic mycoses, blastomycosis, histoplasmosis, and coccidioidomycosis, by a mechanism dependent upon Th17 cells [90]. When examining the contribution of the C-type lectin receptors dectin-1, dectin-2, and mincle to vaccine immunity, the authors found that the specific receptors required for protective responses differed comparing the three causative fungal pathogens. However, the adapter protein Card9 was required for vaccine immunity against all three.

Conclusions

Persons with intact physical and immunological host defenses rarely get fungal infections due to strong innate defenses which link to adaptive immunity. Most of the innate responses are directed against three classes of glycans on the fungal cell wall: β-glucans, mannans, and chitin/chitosan. These glycans serve as danger signals to the host that it might be under fungal attack and an immune response should be mounted. Our laboratory and others are exploring strategies that exploit this finding for use in vaccine development. In this regard, β-glucans appear to be the most promising of the glycans as strong T cell and antibody responses are seen when mice are injected with antigens associated with β-glucans. In contrast, mannans and chitin/chitosan are less immunostimulatory but still may have a role as part of vaccine delivery systems, particularly if administered with adjuvants such as ligands that stimulate TLRs. Finally, fungal glycans tend to be poorly immunogenic. Many studies have examined protection against experimental mycoses afforded by vaccines comprised of protein-carbohydrate conjugates or peptide mimeotopes. A major challenge will be to advance these experimental findings into the clinical arena so that at-risk patients can be protected.

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