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Review

The role of Toll-like receptors and C-type lectins for vaccination against *Candida albicans*

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ABSTRACT

Recent progress has provided important novel insights in the processes driving the adaptive immune responses. Central to these developments is the discovery of pattern recognition receptors like TLRs and CLRs that not only induce innate immune responses, but also modulate cellular and humoral adaptive immunity. As vaccination is one of the great achievements in medicine and probably the most powerful tool to protect human and animals against infectious disease, further vaccine development and optimization of current strategies can improve health status of large groups of people. Development of a vaccine against *Candida* spp. should induce both cellular and humoral immune responses. While the TLRs are strong inducers of inflammatory responses, it seems that the CLRs have the potential to modulate these responses by enhancement or inhibition of cytokine production. Understanding the natural host defense mechanisms against pathogens like *C. albicans* therefore helps to identify the proper targets for inducing a strong adjuvant effect, in order to stimulate an effective adaptive immune response and protection.

Contents

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The incidence of invasive fungal infections has increased over the past decades, due to the augmented use of immunosuppressive therapy, higher incidence of HIV, performance of major surgical procedures and aging of the population [1–3]. *Candida* species are the fourth most common cause of nosocomial bloodstream infections and have a mortality rate up to 40% despite the availability of new effective antifungal agents [4,5]. The current consensus is that antifungal treatments strategies supplemented with immunotherapy, or vaccination in specific patient groups at risk, can improve the outcome and reduce mortality [6,7].

1. The role of innate and acquired immunity for vaccination

Long-lasting protection against microorganisms is gained through the activation of the adaptive immune system represented by cellular immune and humoral immune responses [8]. Depending on type of pathogen and site of infection, an adjusted balance between cellular and humoral responses is desirable [9,10]. Historically, development of many vaccines emphasized induction of the humoral response. However, vaccines against pathogens that cause chronic or mucosal infections should preferable elicit a cellular response [11]. Attenuated or killed microorganisms and pathogen specific proteins or polysaccharide-protein conjugates have been used as vaccination strategies [8]. Three signals are critical for effective stimulation of an adaptive immune response: (a) the presentation of antigens by antigen-presenting cells (APC) on MHC-II to T-cell receptor (TCR) on naïve CD4+ T-cell, (b) the up-regulation of co-stimulatory molecules, and (c) production of cytokines that direct differentiation of a certain type of helper T-cell (Th) response (Fig. 1). This process is induced and regulated by recognition of pathogen associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) on the surface of APCs [12–14]. Depending on the cytokine cocktails induced by PRR activation, the induction of the adaptive immune response is directed towards a Th1, Th2 or Th17 phenotype [15–17]. Optimal inflammatory conditions are induced by immunization with living organisms, while vaccines containing only purified antigens need addition of adjuvants that have to induce stimulation of co-stimulatory molecules and cytokines [11,18]. Most commonly used adjuvants are aluminum and calcium salts which are licensed for human use and enhance the humoral response [19] and they induce their effects through activation of PRRs. For example alum has been shown to exert its effect through recognition by NALP3, a pattern recognition receptor from the NLR family [20,21]. It is also expected that the novel squalene-based emulsion MF59 acts through activation of intracellular innate immune receptors, although the precise mechanism has to be further investigated [22,23]. The deeper insight in the innate immune system obtained in the last years has lead to the design of new adjuvants based on PAMP-PRR interaction, in order to enhance specific cellular immunity elicited by vaccines. One such adjuvant now in clinical trials is the TLR9 agonist CpG-DNA [24].

2. Vaccination strategy against *Candida albicans*

C. albicans is a commensal microorganism, capable of colonizing mucosal sites of humans. Normally this does not lead to disease, because the epithelial barrier protects the host against invasion of fungi. This protection is based on the anatomical barrier and the local immune response, both limiting the fungal burden and leading to a state of tolerance. When the local immune response is insufficient, a high fungal burden at the mucosal site will cause mucocutaneous candidiasis. When the epithelial barrier is breached, for example by surgical procedures or catheters, the local immune response will be crucial to prevent translocation of fungi to the blood stream (candidemia), which can lead to systemic candidiasis when the fungi cannot be cleared. Individuals with an impaired mucosal immune response have and a high local fungal burden and are at higher risk to develop a systemic candidiasis when their epithelial barrier is breached, or their systemic immune response is insufficient (e.g. neutropenia).

The innate host defense and cellular immune responses against *C. albicans* play an important role in the pathogenesis of mucocutaneous candidiasis, as illustrated by the high incidence of *C. albicans* infections in patients with HIV infection and low number of CD4+ T-cells [25,26], or after treatment with immunosuppressive agents like corticosteroids [27]. These clinical facts are strengthened by results obtained in experimental models of candidiasis in which it has been shown that the T-cells lead to protection against mucosal [28,29] and disseminated [30] *C. albicans* infections. The humoral immune response may also play a role in invasive candidiasis, as shown by the antibody response against specific fungal cell-wall components in *C. albicans* infections [31,32]. Opsonization by antibodies leads to enhanced phagocytosis and killing of *C. albicans* by human and murine mononuclear cells in vitro [33–35], while B-cell knock-out mice are more susceptible to experimental systemic, but not mucosal, candidiasis [36]. Interestingly however, there is no higher incidence of fungal infections in human with immunoglobulin disorders [37,38]. Passive immunization and the humoral response after active vaccination induces protection against disseminated infections with *C. albicans* [39,40], but does not seem to play a crucial role in mucosal infections [41]. At this moment there is no active *Candida* vaccine available for humans, and passive immunization with neutralizing or opsonizing antibodies are in clinical trials. An excellent overview of the progress in development of fungal vaccines has recently published [7], but less work has been done regarding the optimal adjuvants to be used for *C. albicans* vaccines.

Different strategies have been used to develop an active vaccine against *C. albicans*. The use of specific cell-wall components of *Candida* like Als1P, Als3P, Hsp90, mannans or enzymes like SAP2 [42–45], led to protection against systemic *C. albicans* infection in mice as measured by decreased fungal outgrowth and improved survival. This response was mainly mediated by cellular immunity [30]. These vaccines were mostly administered subcutaneously (s.c.) and adjuvants were needed to optimize protection. In other vaccination models, primary administration of a low dose of live *C. albicans* [46] or a low virulent *Candida* strain like PCA2 [47,48] intravenously (i.v.) led to protection after reinfection with a high dose of *C. albicans*. Oraly administered or localy administered *C. albicans* in a low inoculum protected mice against reinfection with a higher infectious inoculum, this process was mediated by cellular immune mechanisms [41]. These models provided insight in the type of cellular response needed for protection against *Candida* and provided a proof-of-principle that vaccination against *Candida* is feasible.

3. Type of cellular responses needed for protection against *Candida* infections

Activation of the innate immune system by *C. albicans* induces the production of a variety of proinflammatory cytokines and the expression of co-stimulatory molecules, that in turn mediate specific adaptive immune responses leading to protection against disseminated candidiasis and tolerance at the mucosal barrier. It is generally accepted that induction of a Th1 type cellular response is crucial for the defense against *C. albicans* [49–51]. In contrast, a Th2 cellular response is considered non-protective, as it induces class-switch to non-opsonizing antibody subclasses and IgE [52]. Investigation of the role of Th17 in mediating the immune response has shown that Th17 memory cells are induced by *Candida* hyphae [53,54], and in a murine model IL-17AR knock-out mice had an increased susceptibility to systemic candidiasis [55]. However, deleterious effects of IL-17 inflammatory activities have also been