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Protective effect of antigen delivery using monoolein-based liposomes in experimental hematogenously disseminated candidiasis

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ABSTRACT

We evaluated the potential of a liposomal antigen delivery system (ADS) containing *Candida albicans* cell wall surface proteins (CWSP) in mediating protection against systemic candidiasis. Treatment of bone-marrow-derived dendritic cells with CWSP-loaded dioctadecyldimethylammonium bromide:monoolein (DODAB:MO) liposomes enhanced and prolonged their activation comparatively to free antigen, indicating that liposome-entrapped CWSP were released more sustainable. Therefore, we immunized mice with CWSP either in a free form or loaded into two different DODAB:MO liposome formulations, respectively designated as ADS1 and ADS2, prior to intravenous *C. albicans* infection. Immunization with ADS1, but not with ADS2, conferred significant protection to infected mice, comparatively to immunization with CWSP or empty liposomes as control. ADS1-immunized mice presented significantly higher serum levels of *C. albicans*-specific antibodies that enhanced phagocytosis of this fungus. In these mice, a mixed cytokine production profile was observed encompassing IFN-γ, IL-4, IL-17A and IL-10. Nevertheless, only production of IL-4, IL-17 and IL-10 was higher than in controls. In this study we demonstrated that DODAB:MO liposomes enhance the immunogenicity of *C. albicans* antigens and host protection in a murine model of systemic candidiasis. Therefore, this liposomal adjuvant could be a promising candidate to assess in vaccination against this pathogenic fungus.

Statement of Significance

This work describes the immunomodulation capacity of the previously validated antigen delivery system (ADS) composed by dioctadecyldimethylammonium bromide (DODAB) and monoolein (MO) lipids incorporating the cell wall surface proteins (CWSP) from *C. albicans*. Here, we not only present the ability of this system in facilitating antigen uptake by DCs in vitro, but also that this system induces higher levels of pro-inflammatory cytokines and opsonizing specific IgG antibodies in serum of mice immunized subcutaneously. We show that the ADS are efficient nanocarrier and modulate the immune response against intravenous *C. albicans* infection favoring mouse protection. In sum, we show that the incorporation of *C. albicans* antigens in DODAB:MO nanocarries are a promising vaccine strategy against *C. albicans* fungal infection.

1. Introduction

Vaccines are routinely used to protect against microbial pathogens. They usually contain antigens as surrogates of the disease-causing microorganism or a product of it. Antigens may include weakened or inactivated forms of the target pathogen, or subunits

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that could be constitutive or extracellular molecules [1]. Nevertheless, the use of attenuated pathogens raises several safety issues due to possible reversion of the phenotype or residual virulence. These safety problems may be circumvented by using subunit vaccines. In such case, univalent subunit vaccines may have their effectiveness limited due to antigen variations in the target pathogens. The use of complex antigen extracts, instead of single molecules, in the immunogenic preparations may be a way to overcome this issue [2]. Yet, the effective implementation of subunit vaccines is frequently impaired by insufficient immunogenicity when administered without adjuvant [3,4]. Therefore, selecting an appropriate adjuvant or delivery system is as important as selecting antigen candidates.

Formulating protein antigens into nanoparticles has emerged as one of the most promising strategies to enhance the immune response to vaccine antigens [5–9]. Cationic liposomes are interesting adjuvants that also serve as carriers for the targeted delivery of antigens to immune cells. These liposomes tightly bind negatively charged antigens, which may render soluble antigens into a particulate form thereby increasing their *in vivo* half-life [7,9]. In fact, cationic liposomes were used as adjuvants in several studies, enhancing cell mediated or humoral immunity, as well as delivery systems for drugs, DNA or peptides [5,6,10]. Major limitations behind the fact that as yet no adjuvant based on liposomes has been registered for human use seem to be their stability, manufacturing and quality assurance problems [11]. However, these limitations may be overcome by tuning physicochemical properties like size, charge and hydrophobicity [12–14]. Recently, a direct comparative study showed that cationic liposomes were most efficient for the induction of effector antigen-specific T cells *in vivo*, than poly-(lactic-coglycolic-acid) (PLGA) nanoparticles or the clinically used adjuvants Montanide ISA-51 and SWE, a squalene oil-in-water emulsion [13].

Cationic liposomes composed by surfactant dioctadecyldimethylammonium bromide (DODAB) have been used as carriers in drug delivery studies [8,15] as well as adjuvants in vaccination strategies, displaying higher colloidal stability than aluminum hydroxide and better efficacy in inducing cellular immune responses [16–18]. The main advantage of DODAB as an adjuvant is that it requires a lipid concentration lower than the concentrations traditionally used in liposomal formulations [19,20]. However, these preparations can be physically unstable and therefore the incorporation of different neutral molecules, such as cholesterol, 1-monopalmitoyl glycerol and trehalose 6,60-dibehenate have shown to improve the stability without undermining their adjuvanticity [21]. In previous studies we have demonstrated that monoolein (MO), when incorporated as helper lipid with DODAB, could act as a stabilizer, conferring fluidity to the DODAB nanoparticle liposomes by favoring lipid chain mobility [22]. We have successfully used DODAB:MO as a mammalian cell transfection system and as a nanocarrier for *in vitro* gene silencing [15,23]. In particular, we demonstrated that liposomes formed by DODAB and MO at DODAB:MO (1:2) molar ratio, assembled mainly as positively spherical bilamellar vesicles with some internal structures [24]. In this way, in a recent report, we explored this formulation, DODAB:MO (1:2), and described the development of two liposomal nanoparticle antigen delivery systems (ADS), ADS1 and ADS2, loaded with *Candida albicans* cell wall surface proteins (CWSP) as antigens. These ADS assembled as stable negatively charged spherical nanoparticles with an average particle size of approximately 280 nm, indicating that the CWSP readily associated with the liposomes [25]. This efficient adsorption onto the liposomes and their size, mimicking that of natural pathogens, induced a strong, humoral and cell-mediated immunity when compared with free CWSP [26]. *C. albicans* is an opportunistic human pathogen and is by far the most common cause of fungal invasive infections [25]. Despite the availability of new antifungal agents, candidemia is the fourth most common bloodstream infection in hospitalized patients both in the United States and in many European countries [27–29].

Consequently, antifungal vaccines are currently considered one of the most appealing and cost effective strategies against *Candida* infections [27,30–32]. As far as we know, only two vaccines against *C. albicans* infections have completed Phase I clinical trials, the adhesin-like substance 3 (Als3) with aluminum hydroxide as the adjuvant (NDV-3; NovaDigm Therapeutics) [33], and secreted aspartic protease 2 (Sap2) embedded in a virosomal for adjuvanticity (PEV-7; Pevion Biotech) [34].

In this work, we assessed the effectiveness of the designed DODAB:MO liposomal nanoparticle associated with a *C. albicans* CWSP preparation in inducing protection in a mouse model of systemic candidiasis established by the hematogenous route. Our results showed that these liposomal systems induced strong opsonizing antibody responses and a cell-mediated immune response that allowed a significant protection of infected mice.

2. Material and methods

2.1. Materials

Dioctadecyldimethylammonium bromide (DODAB) was purchased from Tokyo Kasei (Japan). 1-monooleoyl-rac-glycerol (MO), Hanks' balanced salt solution (HBSS), glutaraldehyde, propidium iodide (PI) and DTT were supplied by Sigma–Aldrich (St. Louis, MO, USA). Tris–HCl Buffer was provided by Invitrogen/Molecular Probes (Eugene, OR, USA) and ethanol (high spectral purity) was purchased from Uvasol (Leicester, United Kingdom). Dulbecco's Modified Eagle's Medium (DMEM) was supplemented with 2 mM L-glutamine, (all from Sigma-Aldrich) and 10% heat-inactivated fetal bovine serum (FBS) provided by Lonz (Romania); HEPES-Buffer solution pH 7.5 was provided by VWR International (Radnor, PA, USA) and 1 mM sodium pyruvate by Merck (Frankfurt, Germany). Sytox Green was purchased from Thermo Fisher Scientific (Massachusetts, MA, USA) and propidium iodide (PI) was obtained form Sigma-Aldrich.

2.2. Culture conditions of C. albicans strains

C. albicans strain SC5314 was used for CWSP extraction while *C. albicans* 124A clinical isolate [35] was used for infection experiments. All strains were maintained as frozen stocks in 30% glycerol at −80 °C. When needed, yeasts were obtained from a 2 day YPD agar plate (2% D-glucose, 1% Difco yeast extract, 2% peptone and 2% agar) (w/v) incubated at 30 °C.

2.3. Extraction of yeast CWSP

All procedures used for CWSP extraction were performed in a sterile environment and using apyrogenic solutions. CWSP were released from intact yeast cells by DTT treatment as described previously [26]. The concentrated proteins obtained were stored at −80 °C in aliquots of 100 μg/ml.

2.4. Preparation and characterization of CWSP-loaded liposomes

DODAB:MO based liposomes were prepared using the lipid-film hydration method [36]. Briefly, DODAB and MO, at a DODAB molar fraction (χDODAB) of 0.33, were dissolved in ethanol and mixed in a round-bottom flask. The solvent was removed by rotary evaporation, at a temperature 10 °C above the main phase transition of DODAB (Tm ≈ 44 °C), and liposomes formed after hydration of