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Multifunctional liposomes constituting microneedles induced robust systemic and mucosal immunoresponses against the loaded antigens via oral mucosal vaccination

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ABSTRACT

To develop effective, convenient and stable mucosal vaccines, mannose-PEG-cholesterol (MPC)/lipid A-liposomes (MLLs) entrapping model antigen bovine serum albumin (BSA) were prepared by the procedure of emulsification–lyophilization and used to constitute microneedles, forming the proMLL-filled microneedle arrays (proMMAs). The proMMAs were rather stable and hard enough to pierce porcine skin and, upon rehydration, dissolved rapidly recovering the MLLs without size and entrapment change. The proMMAs given to mice via oral mucosal (o.m.) route, rather than routine intradermal administration, elicited robust systemic and mucosal immunoresponses against the loaded antigens as evidenced by high levels of BSA-specific IgG in the sera and IgA in the salivary, intestinal and vaginal secretions of mice. Enhanced levels of IgG2a and IFN-γ in treated mice revealed that proMMAs induced a mixed Th1/Th2 immunoresponse. Moreover, a significant increase in $CD8^+$ T cells confirmed that strong cellular immunity had also been established by the immunization of the proMMAs. Thus, the proMMAs can be immunized via o.m. route to set up an effective multiple defense against pathogen invasion and may be an effective vaccine adjuvant-delivery system (VADS) applicable in the controlled temperature chain.

1. Introduction

Mucosal vaccines are inoculated at the site of cavity or lumen mucosas which are commonly rich in dendritic cells (DCs) and are, therefore, able to induce both systemic and mucosal immunoresponses not only at the site of antigen exposure but also at remote mucosas due to the existence of generalized mucosal immune network where numerous mucosa-associated lymphoid tissues (MALT) are located [1]. To function effectively, mucosal vaccines must approach the professional antigen-presenting cells (APCs) to induce potent immunoresponses to produce functional pathogen-specific antibodies and cytotoxic T lymphocytes (CTLs), neutralizing and lysing the invaded pathogens. Ideally, a mucosal vaccine would be able to elicit functional immunocytes to secrete mucosal immunoglobulins blocking the establishment of initial infection by the pathogens, such as HIV and HPV, which once enter the cells can rapidly integrate into the host genome to establish a latent reservoir that can hardly be eliminated by conventional antiretroviral agents [2]. Thus, the mucosal vaccines that can block the initial invasion of intractable pathogens are most desirably needed.

However, the mammal mucosas suitable for vaccination are usually covered with a defending layer of mucus which is a continuously renewed viscous fluid and contains various categories of agents, such as antiseptic lysozyme, proteins and glycoprotein mucins [3]. The defending mucus and enzymes impose a potential

Abbreviations: MPC, mannose-PEG-cholesterol; MLL, MPC/lipid A-liposome; proMMA, proMLL-filled microneedle array; BMA, blank microneedle array; VADS, vaccine adjuvant-delivery system; CTC, controlled temperature chain; SPC, soy phosphatidylcholine; MAIM, microneedle array inverse mold; AE, association efficiency; MD, mean diameter; o.m., oral mucosal; i.d., intradermal; s.c., subcutaneous; a.e., anesthesia/anesthetization; BSA-Al, BSA-alum; MALT, mucosa-associated lymphoid tissue; DC, dendritic cell; CTL, cytotoxic T lymphocyte; PEL, procedure of emulsification–lyophilization; OCT, optimal cutting temperature; s.l., sublingual.

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damage to vaccine antigens and prevent mucosal vaccines from approaching and crossing the epithelial layer, under which the professional APCs are most likely sited. Moreover, the tightly-lined epithelial cells with intercellular spaces sealed by tight junctions also form a barrier to mucosal vaccines to reach the professional APCs which are the necessary sponsors for the immunoresponses to the vaccines. By comparison to other sites, oral mucosa confronts a mild environment and is easily accessible and relatively safe for vaccination [4]. The oral mucosa is also rich in APCs and can mediate innate and adaptive immunoresponses to block local and systemic infections [5]. However, oral mucosal immunization is further limited by its anatomic structure of stratified squamous epithelium (SSE) as well as by rapid clearance of the subjects from mucosal surfaces by flow of saliva, movement of tongue and jaws, and chewing and swallowing [6]. To conquer these barriers to oral mucosal delivery, numerous technologies have been developed, including supersaturation, eutectic formation, iontophoresis, electroporation, sonophoresis, laser radiation, photomechanical waves and needleless injection [6]. But a common strategy was to incorporate a bioadhesive component to the drug/carrier to achieve prolonged mucosal contact and higher drug concentration on the mucosal surface [7]. Unfortunately, the bioadhesive component may cause oral paraesthesia or foreign body sensation which accelerates in biofeedback salivation and swallow leading to ingredient loss as well as poor patient compliance; moreover, due to its adherence to the upper epithelia of SSE, bioadhesive component also limits vaccine uptake into the underlying professional APCs resulting in weakened efficacy. Another approach is to use permeation enhancers (e.g., a mucolytic agent of N-acetyl-L-cysteine) to enhance the bioavailability of protein-containing nanoparticles delivered via mucosal route, however, the mucus barrier properties cannot be undermined without compromising the mucosa [8]. Recently, researchers modified nanoparticles with PEG as so-called mucus-penetrating particles (MPPs) for topical delivery of antibiotics in vagina and proved that MPPs improved vaginal drug distribution and retention over the vaginal epithelium compared to conventional particles [9]. But no enhanced uptake of agents into cells of interest can be expected for such an MPP as PEG is a known barrier to the access by most kinds of cells [10].

Previously, we showed that the mannose-PEG-cholesterol MPC/lipid A-liposomes (MLLs) could efficiently carry, protect and present antigens and, therefore, proved an effective oral mucosal vaccine adjuvant-delivery system (VADS) [11]. However, the MLLs also confront the above multiple obstacles which are difficult to overcome with the abovementioned common methods. Recently, microneedle arrays (MAs) with sub-millimeter structures designed to pierce the skin and deliver vaccines in the epidermis or dermis compartments without pain provide an especially attractive option for intradermal delivery [12–15]. Interestingly, some researchers primed mice with intradermal (i.d.) biodegradable microneedles and then boosted by intranasal inoculation with the aqueous formulation, and finally they elicited robust antigen-specific humoral as well as mucosal immunity [13]. Inspired by this, we proposed that microneedle vaccines might as well be administered at oral mucosal sites to penetrate the barriers encountered with conventional mucosal vaccines.

In this report, the MPC/lipid A-liposomes (MLLs) (Fig. 1) entrapping a model antigen, BSA, were also prepared by the procedure of emulsification–lyophilization (PEL) but subsequently used to constitute the microneedles of a biodegradable microneedle array. The proMLL-constituted microneedle arrays (proMMAs) (Fig. 1) were rather stable due to lack of water. When given to mice at oral cavity mucosa, the proMMAs could induce robust systemic and wide mucosal immunoresponses against the loaded antigens. Such a design for the proMMA and its administration eliminate expectedly several substantial obstacles, such as the inability of intradermal microneedles to induce extensive mucosal immunoresponses, the inefficiency of conventional vaccines in penetrating the mucus and tight epithelium of mucosa, and the loss of a large fraction of active ingredients when vaccines gone with mucus fluids and saliva, which are confoundedly encountered in the development of conventional oral mucosal vaccines or microneedle vaccines.

2. Materials and methods

2.1. Materials

Soy phosphatidylcholine (SPC, purity > 97%) was purchased from Lipoid (Ludwigshafen, Germany). Bovine serum albumin (BSA), ovalbumin (OVA), aluminum phosphate (−200 mesh), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), cytochalasin D, stearylamine (SA), monophosphoryl lipid A (LA), IFN-γ and IL-4 assay kits were commercial products by Sigma (Shanghai, China). Goat anti-mouse IgG-horse radish peroxidase (HRP), IgG1-HRP, IgG2a-HRP and IgA-HRP were purchased with sales package of 200 μg per 0.5 mL from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). PE-conjugated anti-mouse $CD8^+$ mAb (monoclonal antibody) and FITC-conjugated anti-mouse $CD4^+$ mAb were biological products of eBioscience (San Diego, USA). The APC surface mannose receptor-binding molecule

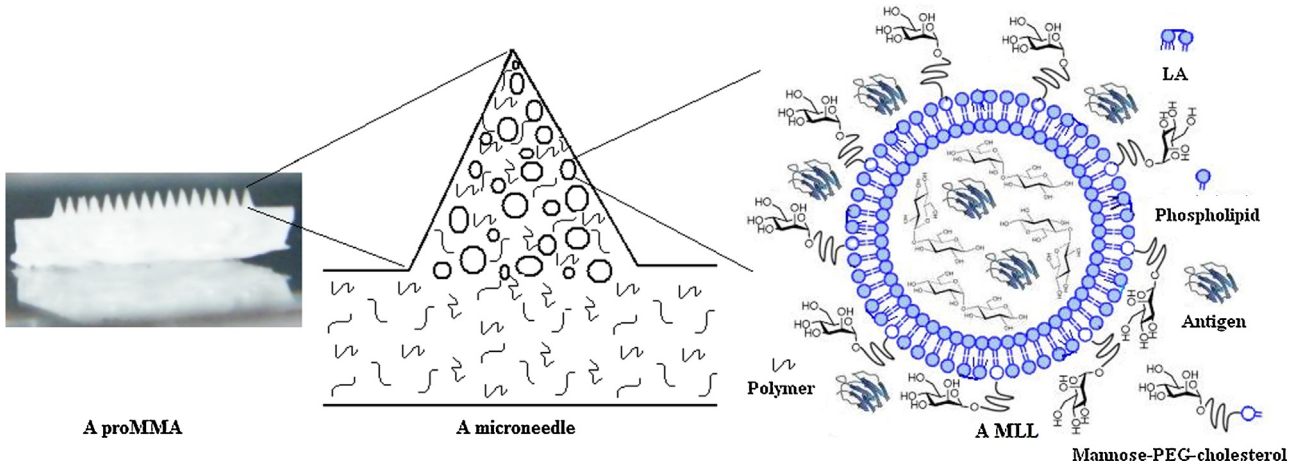

Fig. 1. Structure of the proMMA and MLL.

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