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Galactosylated liposome as a dendritic cell-targeted mucosal vaccine for inducing protective anti-tumor immunity

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ABSTRACT

Mucosal surfaces contain specialized dendritic cells (DCs) that are able to recognize foreign pathogens and mount protective immunity. We previously demonstrated that intranasal administration of targeted galactosylated liposomes can elicit mucosal and systemic antibody responses. In the present study, we assessed whether galactosylated liposomes could act as an effective DC-targeted mucosal vaccine that would be capable of inducing systemic anti-tumor immunity as well as antibody responses. We show that targeted galactosylated liposomes effectively facilitated antigen uptake by DCs beyond that mediated by unmodified liposomes both in vitro and in vivo. Targeted galactosylated liposomes induced higher levels of pro-inflammatory cytokines than unmodified liposomes in vitro. C57BL/6 mice thrice immunized intranasally with ovalbumin (OVA)-encapsulated galactosylated liposomes produced high levels of OVA-specific IgG antibodies in their serum. Spleen cells from mice receiving galactosylated liposomes were restimulated with OVA and showed significantly augmented levels of IFN-γ, IL-4, IL-5 and IL-6. In addition, intranasal administration of OVA-encapsulated beta-galactosylated liposomes resulted in complete protection against EG7 tumor challenge in C57BL/6 mice. Taken together, these results indicate that nasal administration of a galactosylated liposome vaccine mediates the development of an effective immunity against tumors and might be useful for further clinical anti-tumoral applications.

1. Introduction

The mucosal surfaces, such as the gastrointestinal and respiratory tracts, represent the main entry site for most infectious agents. Thus, mucosal immunity provides the first line of defense against harmful microorganisms. The nasopharynx-associated lymphoid tissue (NALT) contains specialized M-like cells, which are structurally similar to those present in Peyer's patches in the gut [1]. The structural and functional role of M cells is thought to lie primarily in antigen uptake and transportation to underlying lymphocytes and antigen presenting cells (APCs) in the mucosal tissues [2]. Mucosal surfaces also contain specialized dendritic cells (DCs). DCs play a critical role in recognizing environmental pathogens, as well as in initiating and regulating adaptive immune responses [3]. Upon capture of antigen, DCs are rapidly activated by a complex process, become mature and migrate into the secondary lymphoid organs, such as the lymph nodes or the spleen, where they present the processed antigenic peptides in the context of either MHC class I or II molecules to CD4 + or CD8 + T lymphocytes, thereby triggering adaptive immune responses [4,5]. In addition, mature DCs also release a variety of cytokines and chemokines to regulate both the innate and adaptive immune responses [6]. Thus, a number of studies have been aimed at developing effective mucosal vaccines to induce protective immunity against cancer and viral infection [7]. Recently, next-generation mucosal vaccines based on synthetic antigens, such as subunit protein, peptide and DNA, have shown potential for the development of safe and effective vaccination strategies [8]. However, due to their weak immunogenicity and low cellular uptake efficacy by

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APCs in mucosal tissue, these synthetic antigen vaccines require multiple or larger doses to reach a satisfactory level of immune protection [9]. Thus, a more effective delivery system is required to enhance the efficacy of mucosal vaccines.

Liposomes are small particulate vesicles formed from phospholipid bilayers that can be used to encapsulate antigen or immunomodulatory molecules. Past studies have reported that liposomes are capable of improving the delivery of antigens across mucosal membranes [10] and enhancing the immunogenicity of various antigens to boost the cellular or humoral immune response [11–13]. Our previous studies also demonstrated that intranasal immunization of inactivated virus encapsulated in a liposome [14] or co-administrated with immunostimulatory molecules [15] can successfully promote efficient mucosal and systemic antibody responses. Recently, surface modification of the targeting ligands and antibodies, which are specifically recognized by receptors on the surface of APCs, has shown the potential to improve the uptake of liposomes and better induce effective immunity against viruses or tumors [16,17].

C-type lectin receptors (CLRs) expressed by DCs are particularly important for the recognition of glycosylated self-antigens or foreign pathogens. CLRs interact with these antigens mainly through the recognition of carbohydrate structures, such as mannose, fucose and glucan, and promote endocytosis, leading to the processing and presentation of antigens on MHC class I and II molecules [18]. Macrophage galactose-type C-type lectins (MGL) in humans and mice are type II transmembrane glycoproteins with the capacity to bind to galactose, N-acetylgalactosamine and Lewis X as mono- or oligosaccharides [19]. It has been shown that the terminal galactose has a high affinity for mouse MGL receptors on murine macrophages [20]. Furthermore, previous studies have identified MGL expression on immature DCs in humans and mice and have shown that MGL mediates the uptake of antigens containing GalNAc residues [21,22]. Thus, galactose is a potent candidate ligand for targeting APCs to induce effective immunity.

We have recently demonstrated that incorporating galactose-1,2-didodecanoyl-sn-glycero-3-phosphoethanolamine (DLPE) into liposomal bilayers as targeted antigen delivery carriers (galactosylated liposomes) resulted in an increase in the uptake and production of cytokines by macrophages [23]. Mice given galactosylated liposomes intranasally showed a significant increase in mucosal secretory immunoglobulin A (s-IgA) and serum IgG antibody responses. DCs are widely accepted as an ideal platform for vaccine design. However, there has been little discussion about the potential of galactosylated liposomes to target DCs. The aim of this study was to evaluate and validate whether galactosylated liposomes could act as an effective DC-targeted mucosal vaccine that could induce systemic antitumor immunity as well as antibody responses. Our results show that galactosylated liposomes effectively facilitated antigen uptake by DCs both in vitro and in vivo. In addition, we established the protective efficacy of ovalbumin (OVA)-encapsulated galactosylated liposomes as a mucosal vaccine against tumor growth.

2. Materials and methods

2.1. Materials

Phosphatidylcholine was purchased from Degussa, Hamburg, Germany. Cholesterol, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), OVA, carbonate–bicarbonate and organic solvents were purchased from Sigma–Aldrich (St. Louis, MO, USA). The galactosyl lipids (alpha-galactosyl-DLPE or beta-galactosyl-DLPE) were prepared by conjugation of galactose with DLPE, as described previously [23]. Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG and IgA were purchased from Bethyl Laboratories (TX, USA). Fetal bovine serum (FBS), penicillin and streptomycin were purchased from Gibco. The mouse cytokine kit interleukin (IL)-4, IL-5, IL-6 and interferon (IFN)-γ, fluorochrome-labeled anti-mouse monoclonal antibodies CD11c, CD80, CD86, major histocompatibility complex class II (MHC-II) and BD Cytofix/Cytoperm Plus (with GolgiPlug) were purchased from BD Biosciences (CA, USA). 3,3′,5,5′-tetramethyl benzidine (TMB) was purchased from Bionovas Biotechnology (Ann Arbor, MI, USA). C57BL/6 female mice (6–8 weeks old) were obtained from the BioLASCO Taiwan Co., Ltd. and acclimated for 1 week prior to the study. All animal experiments were conducted in specific pathogen-free conditions and in compliance with guidelines provided by the Taipei Medical University of Science and Technology for the care and use of animals for research purposes. The E.G7-OVA cell line was obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan) and maintained in complete RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, penicillin (100 U ml^{-1}) and streptomycin (100 μ ml^{-1}) at 37 °C in 5% CO_2.

2.2. Preparation and characterization of liposomes

Liposomes were prepared as described previously [23]. Briefly, thin films were obtained through the rotary evaporation of chloroform solutions with a 4:4:0.5 lipid molar ratio of phosphatidylcholine:cholesterol:galactosyl lipid (alpha-galactosyl-DLPE or beta-galactosyl-DLPE) using a final lipid concentration of 2 mM. The lipid films were maintained under vacuum for 60 min to remove residual organic solvents. Phosphate-buffered saline (PBS) containing 5 mg ml^{-1} OVA was added to the dried lipid film, and multilamellar vesicles were prepared by intense vortex dispersion. The vesicles were extruded 10 times through a 1 μm pore polycarbonate membrane to form galactosylated liposomes (alpha-gal-liposome or beta-gal-liposome). The amount of entrapped OVA was measured by Bio-Rad protein assay, and non-encapsulated OVA was removed by centrifugation. The OVA-encapsulated unmodified liposomes (bare-liposome) were also prepared according to the above procedure. Equivalent quantities of galactose were added to bare-liposome to form a galactose-mixed-liposome (gal-mixed-liposome).

To evaluate the uptake of liposomes by dendritic cells, DiI-labeled liposomes were also prepared by adding 0.04 mol% DiI (percentage of total lipid moles) to chloroform solutions according to the above procedure. Free DiI and DiI aggregates were removed by centrifugation. The particle size and zeta potential of the resulting preparations (200 μl diluted in 4 ml of distilled deionized water) were confirmed by dynamic light scattering analysis using a particle analyzer (BIC 90 Plus, Brookhaven Instruments, Holtsville, NY, USA).

2.3. Generation of bone-marrow-derived dendritic cells (BMDCs)

Mouse BMDCs were generated according to a published protocol [24]. In brief, bone marrow cells were isolated from C57BL/6 mouse femurs and tibias and passed through a 100 μM cell strainer. The red blood cells were lysed using BD Pharm Lyse lysis buffer. The remaining cells were cultured in RPMI 1640 medium (containing 10% heat-inactivated FBS, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin) supplemented with granulocyte macrophage colony-stimulating factor (1000 U ml^{-1}) and IL-4 (500 U ml^{-1}) at 37 °C in 5% CO_2 for 6 days to acquire immature BMDCs. The percentage of CD11c+ cells was verified by flow cytometry, and the cells (final percentage of CD11c+ cells was 85–90%) were used for further in vitro experiments.

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