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Dendrimer-like alpha-D-glucan nanoparticles activate dendritic cells and are effective vaccine adjuvants

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ABSTRACT

The use of nanoparticles for delivery of vaccine antigens and as vaccine adjuvants is appealing because their size allows efficient uptake by dendritic cells and their biological properties can be tailored to the desired function. Here, we report the effect of chemically modified phytoglycogen, a dendrimer-like α-D-glucan nanoparticle, on dendritic cells in vitro, and the utility of this type of nanoparticle as a vaccine adjuvant in vivo. The modified phytoglycogen nanoparticle, termed Nano-11, has a positive surface charge which enabled electrostatic adsorption of negatively charged protein antigens. The Nano-11-antigen complexes were efficiently phagocytized by dendritic cells. Nano-11 induced increased expression of costimulatory molecules and the secretion of IL-1β and IL-12p40 by dendritic cells. Intramuscular injection of Nano-11-antigen formulations induced a significantly enhanced immune response to two different protein antigens. Examination of the injection site revealed numerous monocytes and relatively few neutrophils at one day after injection. The inflammation had nearly completely disappeared by 2 weeks after injection. These studies indicate that Nano-11 is an effective vaccine delivery vehicle that significantly enhances the immune response. This type of plant based nanoparticle is considered highly cost-effective compared with fully synthetic nanoparticles and appears to have an excellent safety profile making them an attractive adjuvant candidate for prophylactic vaccines.

1. Introduction

Vaccines are increasingly formulated with antigens consisting of subunits of microbial pathogens generated through chemical processing or genetic engineering to enhance their safety. Unfortunately, these highly purified vaccine antigens are poorly immunogenic and adjuvants are added to stimulate an effective immune response [1,2]. Adjuvants work, at least in part, by increasing antigen uptake and by promoting the activation of dendritic cells (DCs), a critical step in the initiation of the immune response. The most widely used adjuvants in human and veterinary vaccines are aluminum-containing adjuvants which generally induce a good antibody response, have an excellent long term safety profile, and are relatively inexpensive [3,4]. However, aluminum adjuvants are ineffective in inducing a cell-mediated immune response; are inactivated by freezing; can have a detrimental effect on the stability of vaccine antigens; and are associated with local adverse vaccine reactions. In addition, aluminum is not biodegradable, and most of it is excreted via the kidneys and sweat glands [5,6]. A variety of materials has been tested as vaccine adjuvants with variable degrees of success, and only a few alternatives to aluminum adjuvants have found their way into licensed vaccines [7]. New adjuvants need to stimulate an appropriate immune response, but the single most important consideration is safety [8]. In addition, adjuvants need to be compatible with various antigens, and inexpensive [8].

Over the past 20 years there has been increasing interest in the use of nanoparticles as vaccine adjuvants [9,10]. Immunostimulatory effects have been reported for nanoparticles with a variety of materials, sizes, and surface properties. The physical and chemical composition of nanoparticles affects the type of cells that take up the particles, the trafficking within the cell and the degree and mechanism of immunostimulation [10,11]. Here, we report on the immunostimulatory properties of positively charged phytoglycogen (PG) nanoparticles derived from the kernel of a genetic variant of sweet corn, sugary-1. The lack of a debranching enzyme in this variety of sweet corn results in the formation of dense highly branched, dendrimer-like PG nanoparticles that replace starch granules [12,13]. The surface of PG particles can be chemically modified allowing for functionalization of the particles, such as generating amphiphilic nanoparticles with negative or positive charges. In this study, we investigated the effect of positively

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charged, amphophilic PG nanoparticles, termed Nano-11, on antigen uptake by DCs and activation of DCs in vitro and the adjuvant effect in vaccine formulations in vivo.

2. Methods

2.1. Preparation of Nano-11

The general reaction to prepare Nano-11 is depicted in Fig. 1. Phytoglycogen (PG) was reacted with octenyl succinic anhydride (OSA) to prepare phytoglycogen octenyl succinate (PG-OS), which was further reacted with (3-chloro-2-hydroxypropyl)-trimethylammonium chloride (CHPTAC). Octenyl succinate (OS) groups confer hydrophobicity and negative charges, whereas the quaternary ammonium groups provide positive charges. The final product (PG-OS-CHPTAC) was positively charged by grafting excess amount of CHPTAC groups in comparison with OS groups.

Sweet corn kernels were used as the starting material for PG extraction. The kernels were ground and mixed with six weights of deionized water. The suspension was homogenized and centrifuged at 8000 *g* for 20 min. The supernatant was passed through a 270-mesh sieve. Three volumes of ethanol were added to the supernatant to precipitate polysaccharides. After centrifugation and decanting, the precipitate was suspended using ethanol and filtrated to dehydrate for 3 cycles. The solid material obtained after removing the residual ethanol was PG [14].

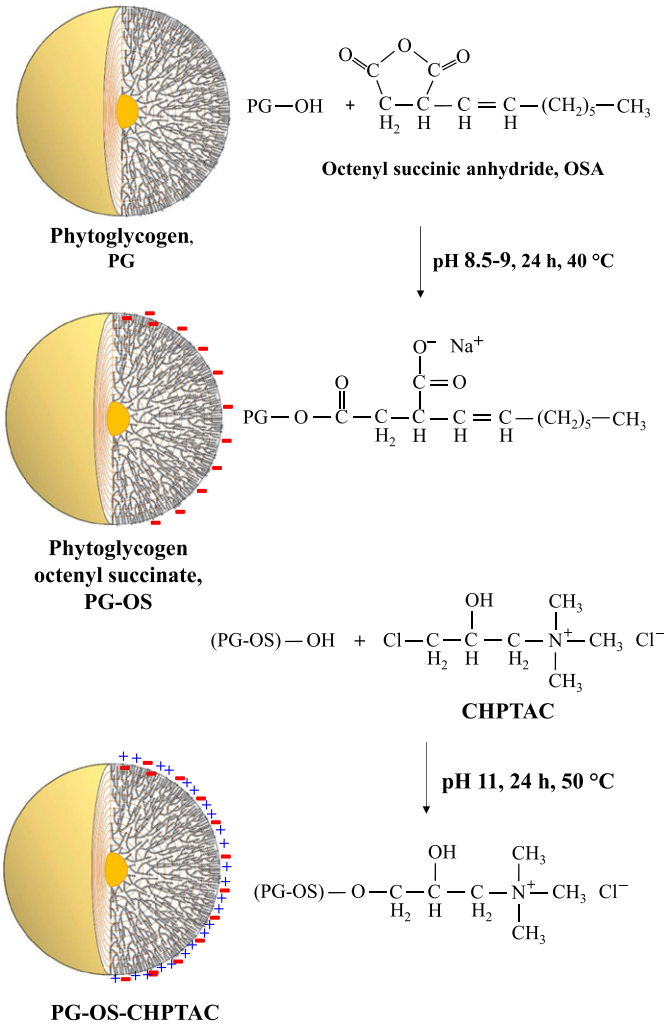

Fig. 1. Preparation of Nano-11 (PG-OS-CHPTAC) from phytoglycogen (PG).

To the dispersion (20%) of PG (20 g of PG in 100 mL water), 1.8 g of octenyl succinic anhydride (OSA) was gradually added in 2 h. The pH was maintained between 8.5 and 9.0 using 5% NaOH. The reaction was carried out at 40 °C and terminated after 24 h by reducing the pH to 6.0–7.0 using 10% HCl. To the dispersion, 3 volumes of ethanol were added, and the precipitate was collected and subjected to 3 cycles of dispersion–filtration using ethanol. The solids obtained were placed in a fume hood to remove residual ethanol. The dry material collected is PG-OS.

The PG-OS material was ground to pass 80-mesh sieve, and 10 g of PG-OS was dispersed in 50 mL deionized water to form 20% dispersion. To this dispersion, 10 mL of CHPTAC was added over a period of 2 h while maintaining pH 11 using 5 M NaOH. The reaction was carried out at 50 °C for 24 h with continuous stirring. Thereafter, the pH was adjusted to 6.0–7.0 using 10% HCl to terminate the reaction. The mixture was precipitated by adding 3 volumes of ethanol and followed by a three-time dispersion–filtration washing procedure using ethanol. The collected solid was placed in a fume hood to remove residual ethanol. The dry material collected is PG-OS-CHPTAC (Nano-11).

2.2. Transmission electron microscopy (TEM)

Droplets of around 0.01% (w/v) PG-OS-CHPTAC in 0.02 M NaAc buffer (pH 5.5) were dried on a 400 mesh carbon-coated grid and stained by 2% aqueous uranyl acetate. Samples were imaged using a Philips CM-100 transmission electron microscope and collected with a CCD camera.

2.3. Zeta potential and particle size

Particle size and Z-potential of nano-11 at pH 2–11 were measured by dynamic light scattering (DLS) using a Zeta-sizer coupled with an MPT-2 titrator (Malvern). Dispersions of Nano-11 were prepared at 1.0 mg/mL and centrifuged at 5000 ×*g* for 10 min. The supernatant was placed in the titrator with constant stirring and automatic titration using 1.0 M HCl as acid, and 0.5 M and 1.0 M NaOH as base. For each of 4 Nano-11 solution samples, 3 readings of Z-average hydrodynamic diameter and zeta-potential were carried out at about a 0.5 interval between pH 2 and 11.

2.4. Generation of bone marrow derived dendritic cells (BMDCs)

Dendritic cells were generated from bone marrow of BALB/c mice as previously described [15]. Analysis by flow cytometry demonstrated that more than 90% of the harvested cells were CD11c+.

2.5. In vitro cytotoxicity assay

BMDCs were resuspended in RPMI-1640 supplemented with 5% fetal bovine serum (FBS) at a concentration of 10^6 cells/mL in a 24-well plate with 1 mL per well. Nano-11 was added at indicated concentrations and the plates were incubated at 37 °C with 5% CO_2 for 2 days. Cell lysis was assessed by a lactate dehydrogenase (LDH) assay kit (Thermo Fisher Scientific, Waltham, MA). The % lysis was determined as the LDH concentration in the sample minus the LDH concentration in cells treated with medium only, divided by the concentration of LDH in cells treated with lysis buffer.

2.6. Adsorption of proteins to Nano-11

Proteins and Nano-11 were mixed in 10 mM 3-(N-morpholino) propanesulfonic acid (MOPS) buffer (pH 7.4) to reach a final concentration for Nano-11 at 1 mg/mL and protein as indicated. The suspensions with a volume of 1 mL were mixed by end-to-end rotation in 2 mL microcentrifuge tubes at room temperature for 1 h. The samples were then centrifuged at 14,000 *g* for 5 min and supernatants were harvested. Protein concentration in the supernatant was analyzed by the