Encapsulation of Antigens into Microparticles Results in Dosage Sparing Capabilities

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Statement of Purpose: Many synthetic biodegradable polymers have been developed as vaccine delivery vehicles and adjuvants. Encapsulating antigens into polymer micro- or nanoparticles is the most common method of formulating these vaccine delivery vehicles. Micro- and nanoparticles can be more easily administered than surgical implantation of discs or tablets because they can be delivered by injection or inhalation [1]. One class of well-studied, biodegradable polymers for drug and vaccine delivery is surface erodible polyanhydrides [2]. These materials possess hydrolytically labile anhydride bonds and have been shown to enhance protein stability [1]. These surface-erodible polymers provide sustained protein release [3], and possess immuno-modulatory capabilities that may influence the characteristics of an immune response [1]. Using a weak immunogen, these studies demonstrated that a more robust immune response was induced following vaccination with antigen-loaded microparticles compared to that induced by soluble antigen alone.

Methods: Ovalbumin (OVA)-loaded microparticles were fabricated using a modified solid/oil/oil (S/O/O) method or cryogenic atomization (CA) [1]. The microparticles were based on 20:80 and 50:50 molar ratios of sebacic acid (SA) and 1,6-bis(*p*-carboxyphenoxy)hexane (CPH). Female C3H/HeNHsd (C3H) mice were immunized subcutaneously at the nape of the neck with 0.5 mg of microparticle formulations (5% OVA-loaded 20:80 CPH:SA and 50:50 CPH:SA for a total antigen dose of 25 μg) suspended in pyrogen-free saline. Separate groups of mice were also immunized with 400 μg or 25 μg OVA alone in 100 μL pyrogen-free saline. Control animals received 100 μL saline alone. Serum samples were collected biweekly over a 12 week period and stored at -20°C until assayed for OVA-specific antibody via ELISA.

Results: OVA-specific serum antibody levels induced by immunization with 50:50 CPH:SA or 20:80 CPH:SA microparticles were elevated in comparison to that induced by a single 25 μg dose of soluble OVA. Mice immunized with 400 μg of soluble OVA were able to elicit a measurable antibody response faster than mice administered microparticles alone; however, vaccination regimens employing OVA-loaded microparticles induced an antigen-specific IgG antibody response that was similar to that induced by 400 μg of soluble OVA through the twelve weeks of the experiment demonstrating at least a 16-fold dose sparing benefit. Significant statistical differences of the microparticle vaccine group titers were only seen from the 20:80 CPH:SA group at 12 weeks in comparison to the 25 μg soluble OVA antigen group (data not shown).

To ascertain the generation of antigen-specific humoral responses, a 25 μg "antigenic challenge" was administered to animals having recieved the OVA-loaded microparticles or soluble antigen 12 weeks earlier. Significant differences were observed in antigen-specific

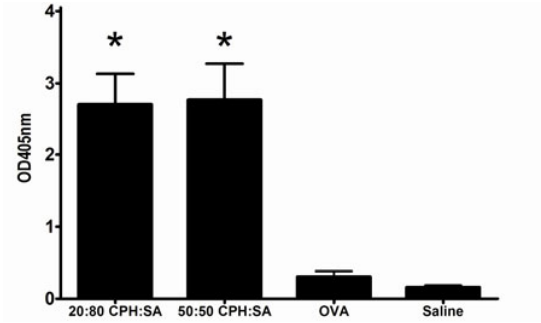

Fig. 1: Twelve week post-injection antigen-specific serum antibody response to Ovalbumin following an antigenic challenge (25 μg OVA) five days prior to serum collection (* indicates $p < 0.05$).

antibody responses in microparticle vaccinated mice versus mice receiving the equivalent soluble dose (Fig. 1).

To further characterize the immune response, OVA-specific IgG1 and IgG2a antibody responses were measured for all of the vaccination groups receiving OVA-loaded microparticles or soluble OVA as the primary dose. The antibody response of mice receiving only the soluble OVA was predominantly IgG1 .

Serum samples collected prior to antigenic challenge were used in an antibody avidity assay [4]. Antigen-specific serum antibody from mice immunized with either OVA-loaded microparticle formulation showed greater avidity for OVA over the 12 weeks post-injection. This evidence suggests that the surface erosion kinetics of the particles allows a sustained release of antigen in vivo that facilitates affinity maturation of the anti-OVA antibody response.

Conclusions: Antigen-specific humoral responses were enhanced to a weak immunogen by encapsulation of the antigen into surface eroding microparticles. In addition, the use of the OVA-loaded microparticles adjuvanted the antibody response and demonstrated dosage sparing capabilities.

References: [1] Kipper MJ. J Biomed Mater Res 2006; 76(4):798-810 [2] Kumar N. Adv Drug Deliv Rev. 2002; 54(7): 889-910. [3] Determan AS. J Control Release. 2004; 100(1): 97-109. [4] Pullen GR. J Immunol Methods. 1986; 86(1): 83-7.