Antigen-Specific Immune Response of Microparticle Vaccine Containing CpG-ODN and Protein

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Statement of Purpose: Protein-based vaccines have great potential in treating cancer and viral and parasitic infections. Since proteins alone generate a weak cellular immune response, they are usually formulated with immunostimulatory molecules to induce a $CD8^+$ cytotoxic T cell immune response. In this study, we investigated the immune response of a vaccine formulated with ovalbumin (OVA) and CpG oligodeoxynucleotide, which were encapsulated in biodegradable microparticles composed of polymer blends of poly(lactic-*co*-glycolic) acid (PLGA) and a co-polyketal PK3 based on 1,4-cyclohexanedimethanol and 1,5-pentanediol [1,2]. Compared to PLGA, PK3 is more pH-sensitive, with a shorter degradation half-life at pH 4.5 than at pH 7.4 [1,2]. CpG is an agonist for TLR9, located in the endosomes of antigen-presenting cells (APCs). CpG stimulates synthesis of IFN-γ to induce a Th1-biased immune response. Thus, we hypothesized that this microparticle vaccine would enhance the Th1-type immune response of a model OVA protein.

Methods: *Microparticle Formulation*: PK3 synthesis followed the method described previously [1,2]. Microparticles were formed using a double emulsion, solvent evaporation method, using 2.5 mg OVA, 0.4 mg CpG, and 100 mg of polymer (either PLGA or a PK3/PLGA 50:50 blend). CpG was ion-paired with DOTAP to form a complex soluble in dichloromethane. Loading of OVA was measured by microBCA, and CpG by OliGreen.

In Vivo: Microparticles in 100 µL volume were injected subcutaneously in C57BL/6 female mice. Mice were boosted 2 weeks later and sacrificed after another 2 weeks. Splenocytes were analyzed for IFN-γ^+ cells (by ELISPOT) and IFN-γ production (by ELISA). Serum IgG antibodies were analyzed by ELISA.

Results: *Formulation:* Microparticles were well-formed, and sizes ranged from 500 nm to 2 µm (**Figure 1**). OVA loading efficiency ranged from 63 to 83% and CpG from 67 to 85%.
Serum antibody response: IgG2b antibodies are enhanced with increasing dose of PK3/PLGA[OVA+CpG] (**Figure 2**). Similar trends were observed for IgG1 and IgG2c isotypes. The microparticles achieved a 5-fold dose-sparing effect compared to soluble OVA + CpG.
IFN-γ production: PLGA[OVA+CpG] and PK3/PLGA [OVA+CpG] each resulted in higher IFN-γ production than PK3/PLGA[OVA] (**Figure 3**), demonstrating the effect of incorporating CpG adjuvant. These results were consistent with an ELISPOT measurement of IFNγ-producing cells (data not shown).

Conclusions: Microparticles co-encapulating CpG and a model protein OVA enhance the antigen-specific Th1-biased immune response. The improved efficacy is attributed to increased uptake of microparticles by APCs and the Th1-polarizing influence of CpG, suggesting a potential formulation for vaccines requiring a $CD8^+$ cytotoxic immune response. Our further research will focus on testing efficacy of this vaccine formulation using antigen proteins, such as NH36 for Leishmania and Tc24 for Chagas disease.

References:

1. Yang SC. Bioconjug Chem. 2008; 19:1164-1169
2. Heffernan MJ. Biomater. 2009; 30: 910-918

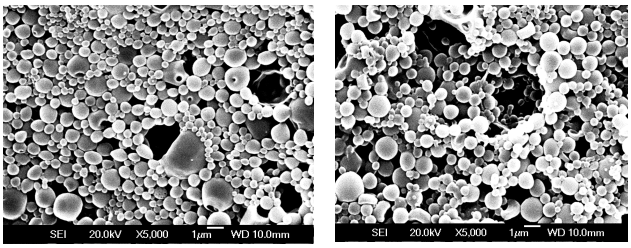

Figure 1. SEM images of PLGA[OVA+CpG] (left) and PK3/PLGA[OVA+CpG] (right).

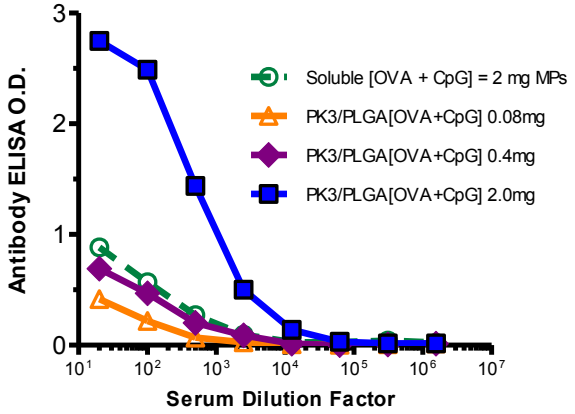

Figure 2. Dose response of mice vaccinated with PK3/PLGA[OVA+CpG]. Serum IgG2b antibody was measured by ELISA (n=5, pooled).

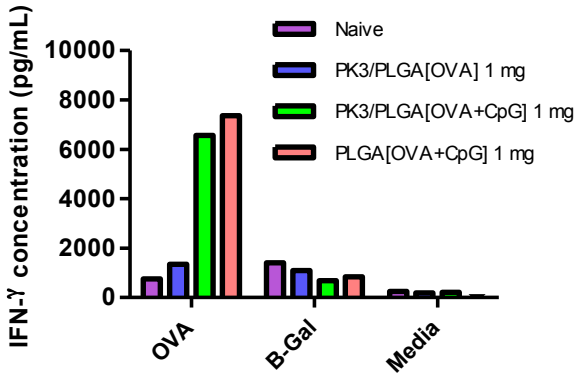

Figure 3. Th1-type immune response in mice vaccinated with microparticle. Splenocytes were stimulated with antigen protein OVA, β-galactosidase control, or media, and IFN-γ was measured by ELISA (n=5, pooled).