Chitosan and Interleukin-12 Enhance the Antigen-Specific T-Cell Response of a Protein-Based Vaccine

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Introduction:
Vaccine formulations that can be easily translated into the clinic are urgently needed. In this study, we investigated the immune response of a vaccine containing ovalbumin (OVA) and interleukin (IL)-12 in a 1.5% chitosan solution. Chitosan is a natural, biodegradable polysaccharide that can be easily mixed with proteins to form an antigen/adjuvant depot at a subcutaneous (s.c) injection site. Viscous chitosan solutions have previously been shown to improve the immune responses of vaccine formulations.[1,2] IL-12 is a pro-inflammatory cytokine capable of inducing a T helper (Th)1 immune response and is considered to have strong potential as a vaccine adjuvant. We thus hypothesized that the chitosan depot would enhance the cell-mediated (Th1-biased) immune response of a model OVA/IL-12 vaccine.

Methods:
Chitosan glutamate (PROTASAN UP G213, NovaMatrix) was dissolved in PBS by vortexing and heating to 60˚C. OVA (A2512, Sigma-Aldrich) and recombinant murine IL-12 (PeproTech) were added at room temperature. The mixture contained 1.5% chitosan, 75 μg OVA, and 0.25, 1, or 4 μg IL-12 in 100 μL injection volume. Controls were prepared with OVA, OVA + IL-12, or OVA + chitosan. C57BL/6 female mice were injected s.c. in the flank on Day 0 and boosted on the contralateral side on Day 14. Mice were sacrificed on Day 21 and splenocytes were analyzed for $CD4^+$ lymphocyte proliferation, cytotoxic T lymphocyte (CTL) function, MHC class I pentamer staining, and interferon (IFN)-γ production. Serum IgG antibodies to OVA were measured by ELISA.

Results:
$CD8^+$ (Cytotoxic) T-cell response: Splenocytes from mice vaccinated with OVA + IL-12 in a 1.5% chitosan solution showed enhanced $CD8^+$ T-cell responses. In a CTL lysis assay, splenocytes from chitosan/IL-12/OVA-vaccinated mice generated 13% killing of EL-4 target cells pulsed with OVA-SIINFEKL peptide (Figure 1) compared to 3% for control peptide-pulsed targets. MHC-I-peptide pentamer staining of mice treated with chitosan/OVA/4μg IL-12 indicated that 1.8% of $CD8^+$ T cells recognized the OVA peptide SIINFEKL versus 0.3% for the corresponding non-chitosan control (Figure 2). In addition, SIINFEKL peptide-stimulated splenocytes generated significantly higher IFN-γ in the chitosan/OVA/4μg IL-12 group than the non-chitosan control.
$CD4^+$ (Helper) T-cell response: The proliferation of $CD4^+$-isolated splenocytes from chitosan/OVA/IL-12-vaccinated mice was higher than OVA/IL-12 and chitosan/OVA controls.
Humoral (serum antibody) response: The chitosan/OVA and chitosan/OVA/IL-12 vaccines resulted in increased levels of total IgG serum antibodies compared to the OVA and OVA/IL-12 controls.

Conclusions:
These results demonstrate that chitosan and IL-12 significantly enhance the antigen-specific $CD8^+$ T-cell response of a model protein-based vaccine. The efficacy of this vaccine is attributed to chitosan's sustained release of the two proteins OVA and IL-12 at the injection site, and the Th1-influencing properties of IL-12. The vaccine is prepared by simple mixing of chitosan, IL-12, and protein antigen. Thus, a clinically relevant vaccine formulation can be readily prepared with a whole protein antigen, such as a tumor-associated antigen or pathogen protein.

References:
1. Zaharoff DA. Vaccine 2007;25:8673-8686.
2. Zaharoff DA. Cancer Res. 2009;69(15):6192-6199.

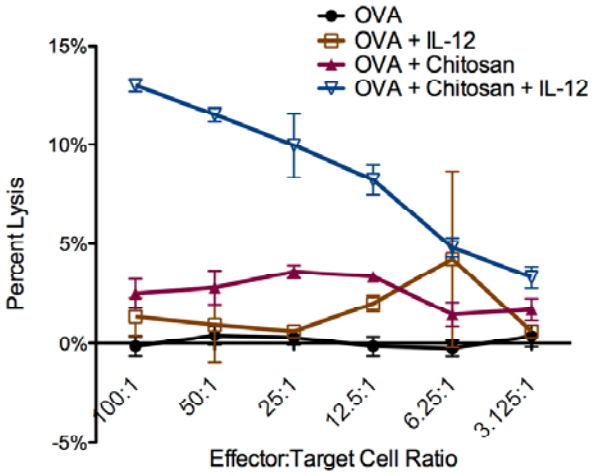

Figure 1. CTL lysis of SIINFEKL-pulsed EL-4 targets by splenocytes from mice vaccinated with 75 μg OVA ± 1 μg IL-12 ± 1.5% chitosan solution (n = 3, pooled).

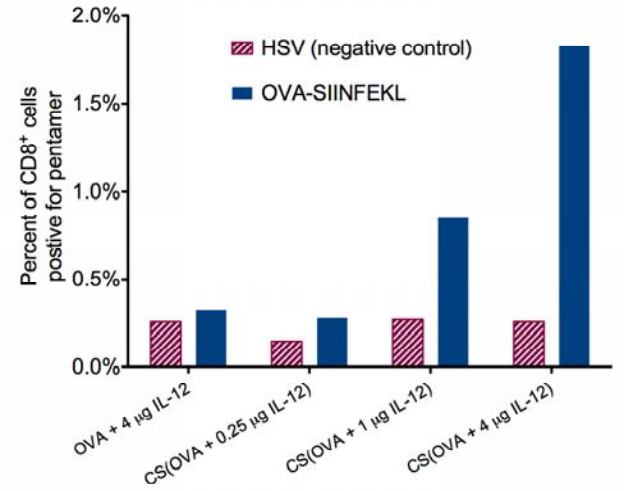

Figure 2. MHC-I-peptide pentamer staining of splenocytes from mice vaccinated with OVA + IL-12 in 1.5% chitosan (CS) solution or in PBS (n = 5, pooled).