In vivo evaluation of immune activation by novel biodegradable polymer adjuvants

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Statement of Purpose: There is growing interest in the development of vaccine delivery systems based on biodegradable polymers because they act as effective adjuvants by providing controlled, programmable sustained release of immunizing proteins, enhanced stability of immunogens and the ability to modulate the immune response[1]. Current adjuvants (alum and MPLA) enhance humoral or Th2-type immunity to delivered antigens. However, the mechanisms by which adjuvants modulate the immune response to specific pathways and establish long term immunologic memory are poorly understood. The ability of a substance to induce antigen-specific T cells of the desired phenotype and to maintain a heightened immune response is crucial to the rational design of vaccines. The purpose of this study was to evaluate the in vivo enhancement and modulation of an immune response to ovalbumin (Ova) encapsulated in novel polyanhydride microspheres.

Methods:

Microsphere Fabrication and Characterization. Several formulations of polyanhydrides based on 1,6-bis(*p*-carboxyphenoxy)hexane (CPH), sebacic acid (SA), and 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) were fabricated by a non-aqueous cryogenic atomization method and characterized by scanning electron microscopy [2,3,4].

Animals. Female DO11.10 TCR transgenic (Tg) mice were purchased from Jackson Laboratories. Prior to adoptive transfer of Tg T cells, DO11.10 mice were euthanized and the spleen and lymph nodes collected[5]. CD4 T cells were purified using a CD4 T cell isolation Kit (Miltenyi). Approximately $2x10^6$ T cells were injected IV into naïve BALB/c mice prior to immunization with Ova alone (2 mg), Ova plus 50 µg LPS, Ova plus 0.5 mg 20:80 CPTEG:CPH microspheres (MS) , LPS alone or MS alone (n=3 or 5/group)[6]. Fourteen days following injection of the Tg T cells, draining lymph nodes were collected and stimulated in vitro with Ova. After 72 h, T cell proliferation and cytokine production were evaluated. At the time of lymph node collection, peripheral blood was collected, serum separated and Ova-specific antibody titers assessed by ELISA as previously described[2].

***Results/Discussion*:** In comparison to cells recovered from control mice, in vitro stimulation of lymphocytes collected from mice 14 days following injection Ova in conjunction with microspheres or LPS showed significant recall responses as measured by proliferation (Figure 1) and cytokines (data not shown). Cultures stimulated with Ova plus MS or LPS yielded SI of 28.8 and 28.2, respectively, whereas Ova alone only yielded an SI of 5.4 (Figure 1). Cytokines production by these cells showed differential expression patterns. Cells from mice receiving Ova alone produced little IL-2 and IL-10, and no detectable IL-4 or IFN-γ. Cells from mice receiving Ova+LPS produced considerable amounts of IL-2, IL-10, IFN-γ and little IL-4. Cells from mice receiving Ova+MS produced IL-2 and IL-10 but no IFN-γ.

Conclusions: CPTEG:CPH microspheres displayed adjuvant activity, as evidenced by increased T cell proliferation, cytokine production, and increased antibody responses to Ova. These materials show promise as single dose vaccine carriers.

References:

[1]http://www.niaid.nih.gov/director/statements/emerging.htm; Sun H. Vaccine. 2003;21:849-855.
[2]Kipper MJ. J Biomed Mater Res. 2006;76A:798-810.
[3]Torres MP. Biomat. 2007;28(1):108-16.
[4]Determan A. Biomat. 2006;27(17):3312-20.
[5]Lutz BL. J Immunol. Meth. 1999;223:77-92.
[6]Pape PA. J Immunol. 1997;159:591-598

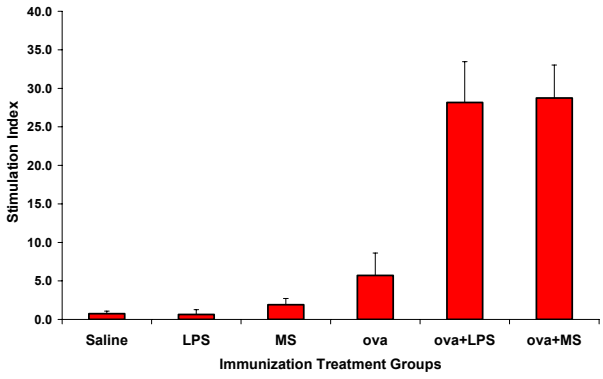

Figure 1. Antigen-specific proliferation of lymphocytes isolated from immunized mice 14 days following passive transfer of Tg DO11.10 T cells. Data is presented as the mean ± SEM of the stimulation index (SI) following the incorporation of ^{3}H-thymidine. n=5.