Humoral responses elicited by polyanhydride nanoparticle formulations are facilitated by enhanced CD4$^+$ T cell helper cells

[1]Lucas Huntimer, [2]Kathleen Ross, [1]Ross Darling, [1]Amanda Ramer-Tait, [2]Balaji Narasimhan, and [1]Michael J. Wannemuehler
[1]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011
[2]Department of Chemical and Biological Engineering, Iowa State University, Ames, IA 50011

Statement of Purpose: Protective immunity elicited by vaccination is often characterized by the presence of high antibody titers, long-lived plasma cells, and memory B cells [1]. Germinal centers (GCs) are anatomically distinct B cell zones where clonal expansion, affinity maturation, and subsequent maintenance of humoral immune responses take place. T follicular helper cells (Tfh) represent a unique CD4$^+$ T cell phenotype that have been shown to play an important role in the generation of GCs and long-lived memory B cells after immunization [2-4]. Nanoparticles based on polyanhydride copolymers of sebacic acid (SA), 1,6-bis(*p*-carboxyphenoxy) hexane (CPH), and 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) provide a means to sustain release of encapsulated protein antigens and to enhance innate immunomodulatory functions associated with dendritic cells and/or macrophages [5-8]. Here, we identify cellular mechanisms important in generation of high titer antibody responses that are elicited by polyanhydride nanoparticle-based vaccine platforms utilizing the ovalbumin transgenic antigen-specific OTII CD4$^+$ T cells.

Methods: Transgenic OTII CD4$^+$ Thy1.2$^+$ T cells were adoptively transferred into Thy 1.1$^+$ mice to assess the clonal expansion of and the development of antigen-specific, memory T cell phenotypes. Mice were immunized with ovalbumin (Ova)-containing CPH:SA and CPTEG:CPH polyanhydride nanoparticle formulations (20:80 C:S, 20:80 C:C, and 50:50 C:C) as well as Ova adjuvanted with Alum or monophosphoryl lipid A (MPLA) or soluble antigen (sOVA) alone. Flow cytometric analysis (FACS) was used to examine expansion of antigen-specific, Thy1.2$^+$ OTII T cells and to monitor effector phenotypes of OTII T cells post-immunization.

Results: The 20:80 C:S and 20:80 C:C nanoparticle vaccine formulations induced significantly greater expansion of Ova-specific CD4$^+$ T cells at 7 days post-immunization as compared to Alum-adjuvanted and sOVA (Fig. 1). OTII T cells recovered from mice immunized with the nanoparticles showed a greater polarization towards Tfh cell phenotype (CXCR5^{high} PD-1^{high}). Ova-specific IgG1 antibody responses were greater with the nanoparticle formulations than sOVA at 14 days post-immunization suggesting that the nanoparticle formulations more rapidly induce Ova-specific GCs.

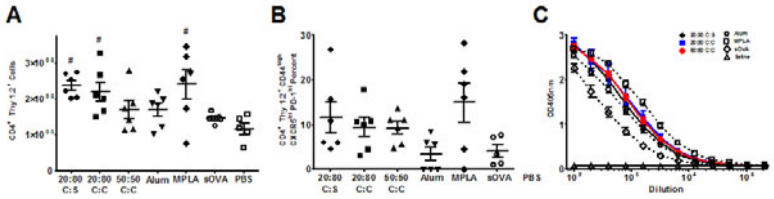

Figure 1. T follicular helper cells, critical for B cell help in generating antibody, are enhanced by polyanhydride nanoparticle-based vaccines. Donor CD4 T cell quantification (A) showing greater expansion of donor OTII CD4$^+$ Thy 1.2$^+$ T cells at 7 days post immunization. (B) Quantification of Tfh phenotype analysis showing gating of CXCR5^{high} PD-1^{high} donor OTII T cells. (C) Day 14 antibody responses examining IgG1 antibody responses. n =5-8 with # indicating $p < 0.05$ from PBS.

Conclusions: Encapsulation of antigen within polyanhydride nanoparticles significantly enhanced the expansion of antigen-specific CD4$^+$ T cells while shifting those cells towards the Tfh phenotype critical for initiating and maintaining germinal centers. This work suggests that an underlying immunological mechanism associated with the elevated antibody responses induced by polyanhydride nanoparticle-based vaccines is, in part, mediated by the induction of antigen-specific Tfh cells.

References:

[1] Murphy K, et al. Janeway's Immunobiology. Seventh ed. New York, NY: Garland Science, 2008: 111-77.
[2] Reinhardt RL, et al. Nat Immunol 2009;10(4):385-93.
[3] King C, et al. Annual Review of Immunology 2008;26(1):741-66. [4]Schwickert TA, et al. Nature 2007;446(7131):83-7.
[5]Carrillo-Conde B, et al. Acta biomaterialia 2010;6(8):3110-9.
[6]Ulery B, et al. Pharmaceutical Research 2009;26(3):683-90.
[7]Torres MP, et al. Acta biomaterialia 2011;7(7):2857-64.
[8]Petersen LK, et al. Biomaterials 2011;32(28):6815-22.
[9]Ulery BD, et al. Sci Rep 2011;1.