In vitro evaluation of immune activation by novel biodegradable polymer adjuvants

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Statement of Purpose: According to the National Institute of Health, infectious disease remains the second leading cause of death worldwide, despite the impressive advances in medical care witnessed over the past few decades[1]. There is a need for the development of novel vaccine delivery systems that can provide controlled release and enhanced stability of protein immunogens (e.g., tetanus toxoid)[2,3,4] with the ability to predictably modulate immune response[2]. Commonly used adjuvants (e.g., alum or MPLA) induce effective humoral immunity by induction of neutralizing antibody. Induction of Th1 type immunity or cytotoxic T cell mediated immunity would enhance the immune response to intracellular pathogens and cancers. Previous studies indicated that vaccine delivery systems based on polyanhydride microspheres have the ability to modulate the Th2:Th1 immune response[2]. Because innate immune mechanisms are central to induction of long-lived immunity, it is essential to elucidate the mechanisms by which adjuvants modulate the induction of immune responses (Th1 vs. Th2). In order to address these challenges, this study evaluated the ability of novel polyanhydride microspheres to stimulate murine dendritic cells (DCs).

Methods:

Microspheres. Polyanhydrides based on 1,6-bis(*p*-carboxyphenoxy)hexane (CPH), sebacic acid (SA), and 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) were used in various ratios. Poly(CPH) and poly(SA) are hydrophobic polymers, which can increase cell uptake of microspheres, whereas the hydrophilic CPTEG is added to maintain stability of the protein. Microspheres were fabricated by a non-aqueous cryogenic atomization method as previously described [2,3,4].

Dendritic Cells. Generation and characterization of bone marrow derived dendritic cells (DCs) obtained from BALB/c mice was performed as previously described by Lutz et al.[5]. DCs (2 x 10^6/ml) were cultured for 24 to 48 h in the presence or absence of lipopolysaccharide (LPS) or microspheres comprising varying formulations of CPTEG:CPH or CPH:SA.

In vitro stimulation of DCs. After incubation in the presence of microspheres or LPS, DCs were collected and assessed by flow cytometry for the surface expression of CD11c, MHC II, CD86 and CD40. In addition, culture supernatants were collected and analyzed for the presence of cytokines (IL-6, IL-10, IL-12, TNFα).

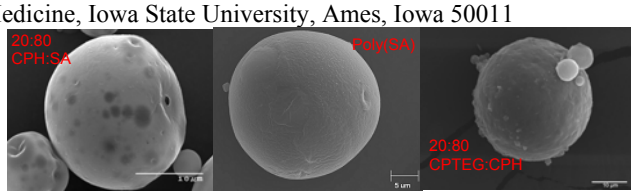

Figure 1. Ova-loaded microspheres representing 20:80 CPH:SA, Poly(SA), and 20:80 CPTEG:CPH.

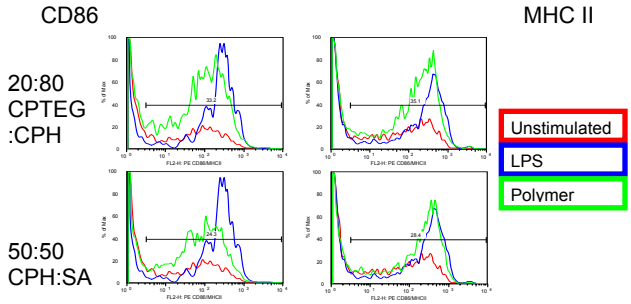

Figure 2. Flow cytometric analysis of DCs cultured in the presence of polyanhydride microspheres.

Results/Discussion:

As shown in Figure 1, microspheres had a smooth surface and are within the size range ideal (<10μm) for phagocytosis by DCs. Following incubation in the presence of LPS or polymer microspheres for 24 h, there was an increase in the number of DCs expressing MHC II and CD86 (Figure 2), and the mean fluorescence intensity (MFI) of these cells as compared to unstimulated DCs. Furthermore, analysis of the cytokines released from DCs incubated with 20:80 CPTEG:CPH and 50:50 CPH:SA showed an increase in IL-12 and IL-6 but not IL-10 or TNFα when compared to unstimulated cultures. While not all polymer formulations activated the DCs equally well, the observed increase in surface marker expression and cytokine production by some formulations suggest that the previously observed adjuvanticity of the polyanhydrides includes the activation of DCs.

Conclusions: The mechanisms underlying the adjuvant activity of polyanhydride microspheres were investigated. In vitro activation of murine DCs was demonstrated by the increased expression of MHC II and CD86 as well as the production of IL-6 and IL-12. These results support and provide a mechanistic foundation for previous in vivo observations that polyanhydride microspheres enhance and modulate the induction of an immune response.

References:

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