Mapping Immune Signatures of Biomaterials
By Christopher Jewell

Introduction
Vaccines have generated one of the largest impacts on human health in history, but there is a rising need for vaccines that are not only potent, but that also allow control over the specific nature of immune response. This idea is termed immunomodulation and refers to the ability to direct immune outcomes, for example, by generating polarized Th1 or Th2 function. Synthetic biomaterials offer opportunities to address immunological questions from new perspectives, and a few biomaterials (e.g., poly(lactide-co-glycolide), PLGA) are now known to exhibit intrinsic adjuvant properties. These polymers trigger “danger”/pathogen sensing pathways (e.g., inflammasomes, toll-like receptors) that control innate and adaptive immunity. Intriguingly, new studies suggest that links exist between structural properties (e.g., hydrophobicity, charge, molecular weight) and adjuvant activity. The goal of this project is to map structural elements of synthetic vaccine carriers that induce distinct sets of cytokines—“immune signatures”. This knowledge could allow design of all-in-one materials that serve both as carriers and adjuvants, and that generate responses tailored for particular diseases.

Aim 1: How do immune signatures vary with polymer structure?

Rationale. We will characterize the immune signatures of nanoparticles formed from libraries of biomaterials in the absence of antigens. These libraries will contain systematic structural changes to vary polymer properties (e.g., hydrophobicity). Nanoparticles will be synthesized by double emulsion from small libraries of PLGA and chitosan, two common biomaterials. Many such polymers are being explored for vaccination because of their ability to be synthesized with designer properties, for example, to degrade and release vaccine components over a desired time scale. Thus, these studies will yield fundamental information about the inherent immunogenicity of an important class of potential synthetic adjuvants.

Approach. Structural perturbations in the polymer libraries will systematically vary physical properties. These synthetic variations include increasing the ratios of lactide:glycolide in PLGA to increase hydrophobicity, and decreasing the extent of acetylation in chitosan to increase cationic charge. Studies will begin in primary cells (APCs, B/T cells), and after pilot investigations, we will conduct in vivo studies in mice using intramuscular injections of nanoparticles (1 mg/kg). For each structural analog, nanoparticles will be administered using equivalent doses, and cytokine analysis will be performed on cells from blood, LNs, and spleen over a 1-week interval. Recent empirical studies have observed induction of a few cytokines by biomaterials, and we will use these observations as initial starting points; however, the real power of this approach is coupling systematic changes in biomaterial structure with multiplexed profiling of many cytokines offered by BD™ Cytometric Bead Arrays (CBAs). Fluorescent antibody conjugates will be used to stain phenotypic surface markers for DCs (CD11b/c), macrophages (F4/80), T cells (CD4/CD8), and B cells (B220). Immune signatures for
each material will then be constructed using CBA kits configured for inflammation (IL-6, IL-10, MCP-1, IFN-gamma, TNF, and IL-12p70) and antibody staining for APC activation (e.g., CD40, CD80, CD86, MHC II). These studies will allow correlation of changes in polymer structure with variation in specific immune function (e.g., does increasing polymer hydrophobicity lead to increased inflammation?).

Aim 2: Can signatures be used to direct immune outcome/efficacy?

Rationale. Aim 2 will introduce antigens into our studies. Immune signatures of free antigens (i.e., without biomaterials) will be determined and compared to signatures of each nanoparticle analog formulated with antigen. The presence of antigen will allow us to track functional (e.g., T cell, B cell/antibody) vaccine response. Aim 2 thus links changes in immune signatures to differences in immunological outcome/vaccine efficacy.

Approach. Studies in Aim 2 will incorporate a well-studied model antigen, ovalbumin (OVA). Following cell treatment/immunization, cytokine signatures will be recorded in parallel with read-outs of immunological functionality in blood, spleen, and LNs. For these experiments, additional CBA panels will be included to evaluate Th1/Th2 function (IL-2, IL-4, IL-5, IFN-gamma, and TNF). T cell functionality will be tested by intracellular cytokine staining following ex vivo re-stimulation with OVA peptides, while ELISA will be used to monitor anti-OVA antibody/B cell response.

Expected Outcomes and Future Extensions

The proposed studies are highly multidisciplinary, linking materials engineering with classical immunology to provide fundamental information of how structural parameters of synthetic polymer carriers activate specific sets of immune pathways (Aim 1). These signatures will also be correlated to functional response when an antigen is present (Aim 2). Our findings could provide insight for both the biomaterials and immunology fields since the work will link immunological mechanisms to fundamental adjuvant characteristics. The “immune signature” aspect of this project underscores the need for multiplexing reagents designed for consistent, multi-parameter cytokine studies. BD CBAs are ideal for these studies, allowing concurrent measurement of multiple cytokines with pre-configured functional panels. The proposed studies and reagents provided through this proposal will also make possible future work focused on clinically relevant antigens (e.g., HIVgag), and broader investigations that evaluate larger cytokine pools (e.g., IL-1beta or other inflammasome indicators). In these future studies we will utilize BD CBA flex sets which allow user-configurable interrogation of up 30 analytes. Taken together, this proposal will generate fundamental information about the interactions between biomaterials and the immune system that could lead to vaccines which offer more precise control over immune response.

The BD Biosciences Research Grant Program aims to reward and enable important research by providing vital funding for scientists pursuing innovative experiments to advance the scientific understanding of disease.

Visit bdbiosciences.com/grant to learn more and apply online.