Multivalent Nanomaterials: Learning from Vaccines and Progressing to Antigen-Specific Immunotherapies

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ABSTRACT: Continued development of multivalent nanomaterials has provided opportunities for the advancement of antigen-specific immunotherapies. New insights emerge when considering the backdrop of vaccine design, which has long employed multivalent presentation of antigen to more strongly engage and enhance an immunogenic response. Additionally, vaccines traditionally codeliver antigen with adjuvant to amplify a robust antigen-specific response. Multivalent nanomaterials have since evolved for applications where immune tolerance is desired, such as autoimmune diseases or allergies. In particular, soluble, linear polymers may be tailored to direct antigen-specific immunogenicity or tolerance by modulating polymer length, ligand valency (number), and ligand density, in addition to incorporating secondary signals. Codelivery of a secondary signal may direct, amplify, or suppress the response to a given antigen. Although the ability of multivalent nanomaterials to enact an immune response through molecular mechanisms has been established, a transport mechanism for biodistribution must also be considered. Both mechanisms are influenced by ligand display and other physical properties of the nanomaterial. This review highlights multivalent ligand display on linear polymers, the complex interplay of physical parameters in multivalent design, and the ability to direct the immune response by molecular and transport mechanisms. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:346–361, 2015

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INTRODUCTION

The use of nanomaterials such as polymers and colloids in medicine has grown dramatically over the past two decades.[1] Although the applications vary greatly, many have explored the ability of these materials to generate an immune response. Nanomaterials can be engineered to have specific characteristics such as size, charge, and shape, properties that influence biodistribution and immune response. Furthermore, current techniques allow researchers to modify nanomaterial display, such as the number, density, and ratio of ligands or antigens on the nanomaterial itself. Although appreciated retrospectively, prophylactic vaccines used to invoke a protective immune response have primarily been colloidal microparticles that have paved the road toward the development of therapeutic nanomaterials.[2] To help explain immune response to nanomaterials, research has continued to explore linkages between nanomaterial characteristics, route of administration, transport, final deposition site, and the resultant immune response.[2,3] In particular, researchers must continue to probe the ability of nanomaterials presenting small molecules, peptides, or other ligands to elicit specific and sustained immune responses not only in the context of vaccines, but also for other immunomodulatory therapies. Finally, new insights for designing multivalent nanomaterial immunotherapies for autoimmune diseases should emerge when considering the backdrop of vaccine design.

Mechanisms of Immune Response

Unlike many other organ systems of the body, the immune system has a unique and vital conscious component in its ability to distinguish "self" (endogenous) and "nonself" (exogenous) antigens. Critical studies reviewed elsewhere have laid the groundwork for identifying mechanisms and communication networks between specialized cell types and resultant immunological responses. In general, a healthy immune system has the ability to act in an antigen-specific manner and can opt to make several decisions after recognition of antigen: recognize antigen as (1) "self" and elicit a nonresponse to that antigen, (2) "nonself" and elicit a nonresponse (generally termed anergy), or (3) "nonself" evoking an immune response against that antigen, potentially leading to immunological memory to that antigen. Specific discrimination between "self" and "nonself" antigens is an essential feature of the immune system. Breakdown in this recognition is thought to be a key player in autoimmune diseases.

One simple model that has helped researchers describe this discrimination phenomenon is the 2-signal model of lymphocyte activation, suggesting the context of antigen presentation helps determine the downstream immune response. In general, interaction of naive B or T cells with antigen is not sufficient to initiate an immune response. It has been proposed that lack of stimulation is a mechanism whereby autoreactive B- and T-cells, which have evaded negative selection processes, are

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prevented from reacting with self-antigens in the periphery, thereby preventing them from causing autoimmune disease. The model proposes that antigen delivered with a secondary activation "context" signal (i.e., costimulation) can evoke a robust immune response toward the offending antigen and can lead to long-term immunological memory. Many of these costimulatory molecules are thought to be mediated by cell:cell interactions of surface receptors (e.g., CD28:CD80, CD40:CD40L).[4–6] Soluble mediators such as innate immune receptor ligands (e.g., lipopolysaccharide, poly I:C) have also been shown to enhance antigen-specific immune response and continue to be actively researched as adjuvants in vaccine formulations.[7,8] Conversely, recent evidence shows secondary signals can also regulate the immune response, suggesting immunological memory can be reprogrammed to elicit an anergic response leading to antigen-specific immune tolerance.[9–11] Although new mechanisms of immune system activation are being discovered at a rapid pace, many identified mechanisms of immune response can be applied to this 2-signal approach to antigen recognition, especially for vaccine formulation strategies classically defined as antigen (signal 1) and adjuvant (signal 2).

Introduction to Vaccines

Vaccines have historically used colloidal suspensions in the 10-μm range to stimulate adaptive immunity and to provide prophylactic protection from infectious diseases.[12,13] The immune system can be primed to prevent and eliminate disease by exposure to a weakened form of the causative pathogen.[14–16] As vaccines have evolved, they have moved toward incorporating safer, more purified pathogen components. Subunit or recombinant vaccines, which deliver only necessary protective antigens, help to eliminate exposure to portions of the pathogen that may cause unnecessary reactivity or harmful side effects. Unfortunately, simple delivery of specific antigen epitopes without immunogenic components (i.e., innate immune system agonists) is often not sufficient to produce long-lasting protective immunity. Therefore, subunit vaccines have been designed to deliver antigen with immunogenic particles, or adjuvants.[17]

Although there is no unified mechanism of action for the array of vaccines currently in the market, the success of colloidal or emulsion-based adjuvants is hypothesized to lie in their ability to (1) enhance and/or stabilize the physical presentation of antigen by acting as an antigen carrier and/or depot, and (2) provide direct stimulatory signals critical for immune cell recruitment and activation. Characteristics of the interaction between adjuvant and antigen such as surface adsorption, changes in protein folding, and antigen epitope stability impact the release of stable antigen as well as the potency and long-term efficacy of a vaccine.[18]

Of the adjuvants approved for human use, the majority have been postulated to form a depot at the injection site.[19] Depots are thought to provide high local concentration of antigen and extend its release over time, allowing for adequate recruitment of immune cells required for establishing long-term immunological memory. Adjuvants also enhance antigen recognition and uptake by making antigen more particulate in nature. Antigens adsorbed to the surface of polydisperse nanometer- to micron-sized aluminum adjuvant particles in suspension, or delivered with oil droplets within an emulsion, fall within a size range comparable to that of a virus or bacteria, and thus more readily undergo phagocytosis by APCs.[14,20] Consequently, portions of, if not entire, viruses and bacteria have been used to create nanoparticles to potentiate immune responses. Intrinsically immunogenic particles such as virus-like particles (VLPs), virosomes, and AS04 provide costimulatory signals for specific innate immune-stimulating receptors.[18,20] For instance, the highly repetitive viral proteins delivered on VLPs can effectively stimulate the pattern recognition receptors of the innate immune system. The adjuvant AS04 combines aluminum phosphate and monophosphoryl lipid A (MPL), a less reactive and less toxic derivative of lipopolysaccharide, to activate toll-like receptor 4 (TLR4) on the cell surface and thereby initiate the TLR4 signaling pathway.[21,22] The use of molecular adjuvants provides the opportunity to more specifically direct immune responses and, in the case of MPL, can help augment the antibody response as much as 100-fold.[23]

Though adjuvants have played a vital role in creating effective vaccines out of safer and more purified antigens, there remains room for improvement. As mentioned above, antigens associated with adjuvants may suffer from unknown or inadequate structure, stability, orientation, or organization. Aluminum adjuvants were commonly thought to form depots; however, there is evidence to suggest otherwise depending on how the antigen associates with the aluminum particle. Antigens within aluminum-adjuvanted vaccines may bind the particle by ligand exchange, especially when the antigen contains phosphate groups, or may be adsorbed to the surface of the aluminum via electrostatic or hydrophobic interactions. Upon contact with interstitial fluid, aluminum begins to degrade and antigen adsorbed to the particle is particularly prone to elute from the aluminum, such that both may diffuse from the injection site. Regardless, aluminum helps maintain the antigen at a high concentration at the injection site while building robust immunity to the antigen, possibly by inducing necrosis and inflammation to attract APCs.[7,13,24,25] Similarly, antigen mixed with MF59 does not incorporate into the oil droplets to any appreciable extent, but is supposedly better recognized within the microenvironment created by the emulsion.[13,26] As our understanding of immune system complexity has improved, the importance of antigen presentation (and even secondary signal presentation) in the appropriate time and space has become apparent. Nanomaterials such as colloids and polymers offer a highly capable delivery system to covalently anchor and more efficiently deliver antigens or ligands directing the immune response in an orientation and pattern that serves to induce desired responses.

MULTIVALENT NANOMATERIALS

Multivalent nanomaterials are rooted in historic vaccine approaches and present new opportunities for restoring immune tolerance. Nanomaterials can be synthesized from diverse raw materials with unique physical and chemical properties well suited for immune modulation and multivalent antigen presentation. Several materials and architectures have been explored as nanomaterial scaffolds for ligand presentation, such as virions, VLPs, linear polymers, polymeric nanoparticles, liposomes, dendrimers, globular proteins, carbon nanotubes, gold nanoparticles (GNPs), and others (Fig. 1). VLPs, for instance, have traditionally been used in nanoparticle vaccines, but have recently