

Minireview: Nanoparticles and the Immune System

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Today nanotechnology is finding growing applications in industry, biology, and medicine. The clear benefits of using nanosized products in various biological and medical applications are often challenged by concerns about the lack of adequate data regarding their toxicity. One area of interest involves the interactions between nanoparticles and the components of the immune system. Nanoparticles can be engineered to either avoid immune system recognition or specifically inhibit or enhance the immune responses. We review herein reported observations on nanoparticle-mediated immunostimulation and immunosuppression, focusing on possible theories regarding how manipulation of particle physicochemical properties can influence their interaction with immune cells to attain desirable immunomodulation and avoid undesirable immunotoxicity. (**Endocrinology** 151: 0000–0000, 2010)

The immune system safeguards the host from infections and malignancies. The immune function is fine-tuned to meet the body's changing requirements for responding to the internal and external environment. The immune system can be perturbed at different levels, resulting in either its suppression or its overstimulation. Therefore, all new chemical and biological entities require adequate investigations into their interactions with the immune system before their use in industry, biology, and medicine (1, 2). Nanoscaled particles can be either engineered or found naturally in the environment (3). Engineered nanoparticles can specifically be designed to either target or avoid interactions with the immune system. An interaction between a nanoparticle and the immune system is considered desirable when it may lead to various beneficial medical applications, such as vaccines or therapeutics for inflammatory and autoimmune disorders (Fig. 1).

Nanoparticle-based delivery systems offer the following potential advantages: 1) site-specific delivery of drugs, peptides, and genes; 2) improved *in vitro* and *in vivo* sta-

bility; and 3) reduced side effect profile. However, because nanoparticles are often first picked up by the phagocytic cells of the immune system (*e.g.* macrophages), there may be undesirable interactions between nanoparticles and the immune system, such as immunostimulation or immunosuppression, which may promote inflammatory or autoimmune disorders, or increase the host's susceptibility to infections and cancer. As mentioned earlier, the main function of the immune system is to protect the host from foreign substances; however, inadvertent recognition of nanoparticles as foreign by the immune cells may result in a multilevel immune response against the nanoparticles and eventually lead to toxicity in the host and/or lack of therapeutic efficacy. For example, granuloma formation was observed in the lungs, skin, and pleural lining of the animals exposed to carbon nanotubes (CNTs) (4, 5).

However, when nanoparticles are recognized as self or there is an absence of immune recognition, this represents a major area of interest in the field of drug delivery. It is now well accepted that properties such as nanoparticle

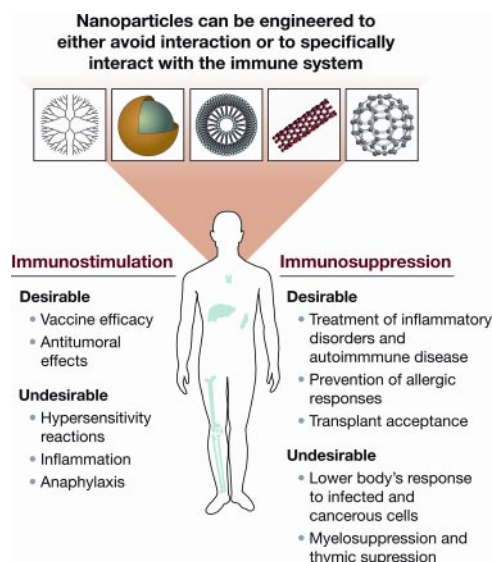


FIG. 1. Nanoparticle interactions with the immune system. Nanoparticles' effects on the immune cells may benefit treatment of disorders mediated by unwanted immune responses and enhance immune response to weak antigens. On the other hand, undesirable immunostimulation or immunosuppression by nanoparticles may result in safety concerns and should be minimized.

size, surface charge, hydrophobicity/hydrophilicity, and the steric effects of particle coating can dictate nanoparticle compatibility with the immune system (6–8). For example, nanoparticles can be designed by attaching to poly(ethylene glycol) (PEG) or other types of polymers to provide a hydrophilic environment, thereby shielding them from immune recognition (9). Although these polymers shield the nanoparticles from recognition by the immune system, there are data suggesting the formation of PEG-specific antibodies after administration of PEG-coated liposomes (10, 11). Consequently, these antibodies resulted in accelerated clearance of the PEG-liposomes from blood and contributed to the change in pharmacokinetic profile of subsequent injected doses of PEG-liposome (12–14). Therefore, it should be noted that generation of particle-specific antibodies may affect the efficacy and the safety of nanoparticle-based therapeutics. Nanoparticles can also be designed to elicit an immune response by either direct immunostimulation of antigen-presenting cells or delivering antigen to specific cellular compartments (15).

The basics of the immune recognition of nanoparticles are reviewed elsewhere (6–8) and are omitted from this discussion. The focus of this review was on the most recent advances in understanding nanoparticle interactions with various components of the immune system. This review encompasses the current knowledge about nanoparticle-mediated immunosuppression and immunostimulation, and gives an overview of development strategies of nanoparticles in different preclinical and clinical phases.

Immunosuppression

Immunosuppression may be either inadvertent or desirable. On the one hand, immunosuppression may lower the body's defense against infection and cancerous cells, and on the other hand, it may enhance the therapeutic benefits of treatments for allergies and autoimmune diseases and prevent rejection of transplanted organs. Although traditional toxicology studies focused on the undesirable consequences of immunosuppression (16), such studies are sparse for nanoparticles. To date, most studies focused on the inflammatory properties of nanoparticles. One of the few studies on immunosuppression has demonstrated that inhalation of CNTs suppresses B cell function and that the TGF- β produced by alveolar macrophages is a key element in the mechanism of the observed immunosuppression (17). Other studies have shown that nanoparticles can be used to deliver immunosuppressive drugs and prevent immunosuppressive properties of small-molecule drugs (18). For example, poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles were administered iv to deliver glucocorticoids to the inflamed joints in the mouse model of arthritis. Complete remission of inflammatory response was achieved with PLGA nanoparticles, and the improved efficacy was due to the targeted and controlled release of steroids from PLGA nanoparticles (19). Another example is a Food and Drug Administration-approved chemotherapeutic, Abraxane, a nanoparticle colloidal suspension in which paclitaxel is bound to human serum albumin nanoparticles. In patients with breast cancer, paclitaxel nanoparticle formulation resulted in a lower incidence of grade 4 neutropenia (a form of myelosuppression that leads to the decreased number of neutrophils) than the first generation of paclitaxel formulation, Taxol, which contained the excipient Cremophor EL (18, 20). Yet another example consists of liposomes loaded with clodronate, which were used in a swine model to eliminate macrophages and thus protect animals from lung injury caused by endotoxin (21).

Immunosuppression is often mediated by toxicity of substances to T cells. For example, some immunosuppressive agents (such as tetrachlorodibenzo-*p*-dioxin, cadmium, corticosteroids, and radiation) have been shown to act against T cells, impairing their development and function (22, 23). Whereas cadmium's suppressive effects on the thymus are well known (23), no studies have been reported regarding the effects of cadmium-containing nanoparticles (*e.g.* quantum dots) on the thymus. Clearly more studies are necessary in this field.

Induction of immune tolerance by nanoparticles can be considered a form of desirable immunosuppression. For example, a water-soluble fullerene derivative (polyhy-

droxy C60) has been shown to inhibit type I hypersensitivity reactions to allergens, both *in vitro* in human primary mast cells and basophils and *in vivo* in a mouse model of anaphylaxis (24). Similarly, allergen-loaded PLGA, chitosan, poly(lactic acid), poly(methyl vinyl ether-co-maleic anhydride) nanoparticles, and dendrosomes have been reported as effective suppressors of type I and type II allergies to environmental and food allergens in animal models and *in vitro* (25–29). Recently it has also been shown that synthetic peptide dendrimers may block experimental allergic encephalomyelitis (30).

Whereas the body of knowledge on nanoparticles is still limited, to date, there are no studies linking the incidence of autoimmune diseases to exposure to nanoparticles. However, a few studies have proposed the use of nanoparticles to treat autoimmune diseases (31). For example, collagen type II entrapped in PLGA nanoparticles can suppress inflammation in a mouse model of rheumatoid arthritis. Likewise, delivery of IL-10-encoding DNA by nanoparticles was successful in the suppression of autoimmune diabetes in mice (31, 32). Treatment of experimental autoimmune uveoretinitis with betamethasone-poly(lactic acid) nanoparticles, and treatment of experimental autoimmune thyroiditis with cytotoxic T lymphocyte-associated antigen-4Ig-silica-nanoparticles in canine models was shown to ameliorate the course of both diseases (33, 34).

Immunostimulation

Nanoparticles are evaluated for their immunostimulatory potential based on their ability to stimulate innate or adaptive immune responses. In the following section, we will describe reported studies on the effects of nanoparticles on the complement system, cytokine secretion, and the induction of antibody response (immunogenicity).

Properties of nanoparticles, which have important interactions with the complement system, have been previously reviewed in detail (7). Briefly, activation of the complement cascade can be harmful if particles inadvertently, or by design, enter the systemic circulation because this may lead to hypersensitivity reactions and anaphylaxis (35–38). Recent data also suggested that activation of the complement system at tumor sites stimulates tumor-associated immune cells and promotes their conversion into a tumor-supportive phenotype, thereby stimulating cancer progression (39, 40). This type of response may impact the therapeutic efficacy of nanoparticle formulations intended for cancer diagnosis or therapy. On the other hand, if particles are intended for sc or intradermal administration, activation of the complement by the particles can benefit vaccine efficacy. Some of these findings have re-

ceived increased attention among the rapidly growing nanomedicine community (40, 41). Whereas many questions still remain, it is important to elucidate how complement activation relates to nanoparticle toxicity and the development of nanotechnology-based formulations for medical applications.

Nanoparticle immunogenicity is drawing interest because nanoparticles have been shown to improve antigenicity of conjugated weak antigens and thus serve as adjuvants and because some nanoparticles have been shown to be antigenic themselves. The former property has been shown to depend on particle size and surface charge (42–44) and can significantly contribute to the development of improved vaccine formulations. Particle size has been reported as a major factor in determining whether antigens loaded into nanoparticles induce type I (interferon- γ) or type II (IL-4) cytokines, thereby contributing to the type of immune response (45). A leading hypothesis on why nanotechnology-driven formulations are effective in vaccine development is that nonsoluble nanoparticles provide controlled, slow release of antigens, creating a depot at the site of injection and providing protection in the destabilizing *in vivo* environment (46, 47).

Antigenicity of the nanoparticles *per se* is less well understood. Two studies demonstrated the generation of particle-specific antibodies when C60 fullerene derivatives conjugated to a protein carrier, BSA, were used for immunization (48, 49). However, other studies using different fullerene derivatives, gold colloids, and cationic polyamidoamine and polypropyleneimine dendrimers have reported no particle-specific immune response, even in the presence of strong adjuvants (50–55). However, polyamidoamine dendrimers conjugated to BSA showed increased antigenic properties and as result dendrimer-specific antibody was observed *in vivo* (56). Common features of all of these studies in which nanoparticle-specific antibodies were generated is nanoparticle conjugation a protein carrier BSA. Therefore, these limited data may suggest that some water-soluble nanoparticles may behave as haptens, *i.e.* they are not antigenic until they bind to protein carrier (Fig. 2) possibly as a result of their small size.

Another area of nanoparticle-mediated immunostimulation is the elicitation of allergic reactions. A few studies linked exposure to nanoparticles to allergic reactions in test animals and humans (57, 58). For example, both single-wall and multiwall CNTs enhanced the allergenicity of egg albumin when administered via intranasal or sc routes in mice. The mechanism of this enhanced allergenicity is thought to be due to the CNT-mediated induction of the acute inflammatory response (57). Occupational exposure during the manufacture of nanomaterials has been

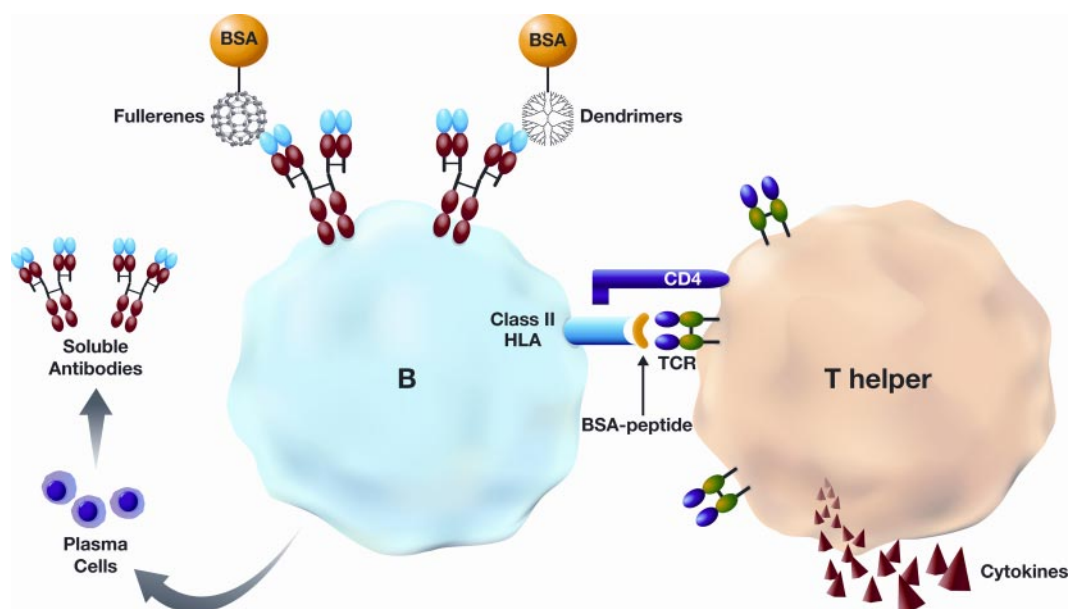


FIG. 2. Hypothetical model of nanoparticle antigenicity. Nanoparticles (dendrimers and fullerenes) may not be antigenic unless they are bound to a protein carrier such as BSA. BSA-nanoparticle conjugate is endocytosed by B cells, and the protein is digested inside the cell. B cells then may present BSA peptides on their human leukocyte antigen-class II molecules to T-helper cells. Both cells become activated, resulting in production of cytokines by T lymphocytes and antibodies directed to nanoparticles by plasma B cells. The protein carrier is indispensable for T cell activation. Antibodies to fullerenes and dendrimers are produced by different B cells. For simplicity of the figure, only one B cell is shown. TCR, T cell receptor.

linked to allergic reactions. For example, toxic epidermal necrolysis-like dermatitis was observed in a chemist exposed to high levels of intermediate or final products of dendrimers while performing dendrimer synthesis (58). However, it is unclear whether the dermatitis was caused by nanoparticles or reactive species used in their synthesis. It should be noted that these studies dealt with raw nanomaterials, which were not intended for biological or medicinal use. More studies involving well-characterized nanoparticles, relevant animal models, and routes of nanoparticle administration are required to understand whether nanoparticles can cause allergy in humans.

In some cases, reformulation of an approved drug in nanoformulation may circumvent allergic reactions associated with the previously approved formulations. Abraxane is an example of such a reformulation. In this case, reformulated paclitaxel-bound albumin nanoparticles did not exhibit any allergic reaction, whereas the first-generation formulation of paclitaxel in the nonionic surfactant Cremophor EL caused severe hypersensitivity reaction, often requiring premedication with a histamine blocker and steroids (59).

Many immunostimulatory reactions initiated by nanoparticles are mediated by the production of inflammatory cytokines. Several studies have reported cytokine induction by different types of nanomaterials (gold colloids, dendrimers, polymers, lipid nanoparticles, *etc.*) (44, 45, 60–63). Nanoparticle size has been suggested as a leading parameter that determines a nanoparticle's potential to induce cytokine responses (44, 45, 60). For example, the

length of CNTs was shown to correlate with sc inflammation induced by CNT *in vivo* (64). However, a few studies have shown that cytokines were induced not by nanoparticles *per se* but by surfactants or bacterial endotoxins present in the formulation (61, 63). Therefore, it is important to quantify the presence of chemical (formation of byproducts) and biological (endotoxin) contaminants before analysis of inflammatory properties of nanoparticles.

Examples of Known Parameters for Interaction with the Immune System

In this part of the review, examples are provided for various nanoparticle platforms currently being developed for drug applications. A brief discussion is provided for each nanoparticle platform's key components and their interaction with the immune system.

Polymeric nanoparticles

Polymeric nanoparticles are defined as nanoscale drug delivery platforms assembled by, for example, biodegradable polymers, dendrimers, and micelles. Polymeric biodegradable nanoparticles have been explored as vaccine formulations, in which antigen is encapsulated in polymers such as PLGA or polylactide. The use of PLGA or polylactide in vaccine development is beneficial because of their biodegradability and biocompatibility (65). Similar to other drug delivery systems, biodegradable polymeric-

based delivery systems such as PLGA may offer the following advantages: 1) they provide sustained release; 2) they protect encapsulated antigen from harsh environment and enzymatic degradation; 3) they provide targeted delivery with attachment of ligands; and 4) they may have adjuvant effects. One of the design strategies to increase the functionality of the nanoparticles is to change their physicochemical properties, such as particle size. For example, it has been reported that smaller nanoparticles (~25 nm) travel through the lymphatic more readily than the larger particles (~100 nm) and accumulate in lymph node resident dendritic cells (43). In addition to size, other physicochemical properties, such as charge, can be manipulated for desired therapeutic benefit.

Nanoliposomes

Nanoliposomes are defined as liposomes in nanoscale assemblies that are composed of one or more bilayers of amphipathic lipid molecules enclosing one or more aqueous compartments (66). From immunological prospective, two types of nanoliposomes have been reported: 1) liposomes designed to elicit immune response to an antigen that is encapsulated in the liposome; and 2) liposomes with a polymer coat to prevent immune recognition. Several liposomal formulations have already been approved for treatment of cancer, infections, and meningitis (67). Many other liposome-based formulations are being developed as therapeutic vaccines against malaria, HIV, hepatitis A, influenza, prostate cancer, and colorectal cancer (68). For example, Stimuvax, a cancer vaccine that targets cancer cells expressing mucin-1 protein, is undergoing phase 3 trials in more than 30 countries including the United States in patients with unresectable stage III non-small-cell lung cancer (<http://www.oncothyreon.com/clinical/stimuvax.html>). It has been hypothesized that the inherent ability of antigen-presenting cells to sequester nanoscale liposomes more efficiently than larger-sized liposome counterparts may be the key to the enhanced immune response observed with nanoliposome formulation. Additionally, the surface charge on nanoliposomes can be manipulated to improve antigen delivery; for example, cationic liposomes are much more potent in generating immune response than anionic or neutral liposomes (69). Targeting moieties such as antibodies can also be attached to liposomes to improve antigen delivery to dendritic cells (70).

Nanoemulsions

Nanoemulsions are emulsions with droplet size in the nanometer scale. An emulsion is a thermodynamically unstable system, unless stabilized by the presence of emulsifying agent, consisting of at least two immiscible liquid

phases, one of which is dispersed as globules (the dispersed phase) in the other liquid phase (the continued phase) (71).

Emulsions are traditionally used in vaccines. It has been reported that the nanoscale-sized emulsions are able to permeate the nasal mucosa and carry the antigen to the antigen-presenting cells more efficiently than larger-sized emulsions. Nanoscale emulsion-based intranasal vaccines have been investigated for hepatitis B, HIV, influenza, and anthrax (72). One example of such an application is a nanoemulsion-based seasonal influenza vaccine NB-1008, currently undergoing phase 1 clinical trials in the United States (http://www.nanobio.com/nanobio_phase1_flu.html). Another application of the nanoemulsion platform is a hepatitis vaccine herein referred to as nano-HBsAg. This vaccine is composed of a 400-nm oil-in-water emulsion containing surface antigen (HBsAg) of recombinant hepatitis B virus. *In vivo* studies showed a robust and systemic IgG, mucosal IgA, and strong antigen-specific immune response after an intranasal administration of the nano-HBsAg formulation (72). Although the serum IgG titers induced by this formulation were comparable with those of an alum-based traditional vaccine, the nanoformulation was more efficient and required fewer administrations. Other advantages of the nanoformulation are the ease of administration, via the nasal route, and a longer shelf life at elevated temperatures, which allows this formulation to be stored at room temperature, overcoming common limitations in developing countries, such as access to a refrigerator (72).

Solid lipid nanoparticles (SLNs)

SLNs are defined as colloidal particles of a lipid matrix solid at physiological temperature (73). Similar to the aforementioned drug delivery systems, SLNs have been used to encapsulate either hydrophilic or hydrophobic molecular entities and generally exhibit particle size of up to 400 nm. When compared with free (not encapsulated) antisense oligodeoxyribonucleotide G3139, SLN-encapsulated G3139 demonstrated greater immunostimulatory property and antitumor activity. It is hypothesized that the increased activity of nanoparticles is due to their small particle size, leading to the more efficient uptake of SLNs by tumor-resident macrophages and dendritic cells (74). Differences in the pharmacokinetic profile of the free *vs.* encapsulated G3139 may also be responsible for the enhanced immunostimulatory effect observed.

Targeted delivery systems

Targeted delivery systems include any delivery platform with a targeting moiety such as antibodies, peptides, and glycoprotein to improve the efficiency and specificity of drug delivery. A targeted nanoparticle-based gene de-

livery concept was translated from bench to bedside for the first time with Rexin-G and Reximmune-C formulations. Rexin-G and Reximmune-C are targeted multilamellar vesicle-based nanoparticles approximately 100 nm in size. The former encapsulates a construct encoding a dominant-negative mutant of human cyclin G1 protein; the latter carries a cytokine, granulocyte-macrophage colony-stimulating factor (75–77). After receiving orphan drug status from the Food and Drug Administration in 2003, the Rexin-G formulation entered phase 1/2 clinical oncology trials in 2007 (www.epeiusbiotech.com). The clinical trials of Reximmune-C formulation started in February 2009 in the Philippines and included a limited number of patients who benefited from previous Rexin-G therapy. These two drug therapies exemplify the two-tier complementary approach aimed at both tumor eradication and cancer vaccination. The first step involves administration of Rexin-G to target and kill the tumor cell in a programmed fashion, whereas the second step employs Reximmune-C to induce a localized cancer autoimmunization (75–77).

Conclusion and Lessons Learned

In summary, existing studies have demonstrated that nanotechnology offers many advantages, such as improved stability, favorable biodistribution profiles, slower drug release kinetics, lower immunotoxicity, and targeting to specific cell populations. Lessons learned from previous studies include the importance of detection and prevention of potential particle contamination with such things as bacterial endotoxins and/or toxic synthesis by-products, and the importance of understanding how route of administration and particle biodistribution in the body may result in either desirable and undesirable immunomodulation (*e.g.* complement activation on iv administration is not desirable, whereas on sc administration, it is beneficial for vaccinations).

Nanotechnology platforms are being investigated as vaccine carriers, adjuvants, and drug delivery systems to target inflammatory and inflammation-associated disorders. Some formulations are already in clinical trials, whereas many others are in various phases of preclinical development. Although in recent years, our understanding of nanoparticle interaction with components of the immune system has improved, many questions still require more thorough investigation and deeper understanding. Further mechanistic studies investigating particle immunomodulatory effects (immunostimulatory and immunosuppression) are required to improve our understanding of the physicochemical parameters of nanoparticles that define their effects on the immune system.

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