Minireview

Nanoparticles and direct immunosuppression

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Abstract

Targeting the immune system with nanomaterials is an intensely active area of research. Specifically, the capability to induce immunosuppression is a promising complement for drug delivery and regenerative medicine therapies. Many novel strategies for immunosuppression rely on nanoparticles as delivery vehicles for small-molecule immunosuppressive compounds. As a consequence, efforts in understanding the mechanisms in which nanoparticles directly interact with the immune system have been overshadowed. The immunological activity of nanoparticles is dependent on the physiochemical properties of the nanoparticles and its subsequent cellular internalization. As the underlying factors for these reactions are elucidated, more nanoparticles may be engineered and evaluated for inducing immunosuppression and complementing immunosuppressive drugs. This review will briefly summarize the state-of-the-art and developments in understanding how nanoparticles induce immunosuppressive responses, compare the inherent properties of nanomaterials which induce these immunological reactions, and comment on the potential for using nanomaterials to modulate and control the immune system.

Keywords: Nanoparticles, immunosuppression, bionanoscience, nanotoxicology, immunotoxicity, anti-inflammatory

***Experimental Biology and Medicine* 2016; 241: 1064–1073. DOI: 10.1177/1535370216650053**

Introduction

Immunosuppression is the effect of a drug or other material to reduce the activation or efficacy of the immune system. Conventionally, immunosuppression is considered an immunotoxic effect implicated in an array of human pathologies. Immunosuppressive therapies often rely on the use of small molecule medications and biologics, and although the acute efficacy of these therapies has improved over the recent decade, long-term use of an immunosuppressant results in co-morbidities due to systemic toxicity or immunodeficiency.[1–3] Immunosuppressive strategies that pin-point specific tissue or pathways, while reducing systemic side effects, are a potential panacea for treating autoimmune disorders and complementing pharmaceuticals. To achieve these goals, many chemical and biomedical researchers have engineered nanoparticles to carry and locally deliver immunosuppressive agents.[4–10] This can be considered "indirect immunosuppression," where the nanoparticle solely serves as the delivery vehicle.[7] As an alternative, a small but prevailing body of literature is reassessing direct immunosuppression by nanoparticles to be exploited as a compliment for drug therapies or organ/tissue transplantation that otherwise would be rendered ineffective or rejected by the native immune response.[11–15] Reports in the fields of nanotoxicology and nanomedicine are exploring the fundamental mechanisms for both intended and unintended immunosuppression by nanomaterials.[16–19] Understanding the direct immunosuppressive effect of nanoparticles and resultant development of new immunosuppressive therapies can impact both pharmaceutical and regenerative medicine technologies.[9,20,21]

When developing methods to suppress immune response, it is important to consider both innate and adaptive immune responses. Innate immunity involves the first response activation of macrophages and neutrophils that secrete specific cytokines, which produce local inflammation at the site of reaction. This inflammation is usually followed by the adaptive immune response that activates antigen specific cells, T cells and B cells to target the pathogen or other instigator of the immune response. Both innate and adaptive responses are intertwined, and responsivity to any immunosuppressive agent is highly dependent on which pathway in the immune system is affected.[22] For the purposes of this discussion, both suppression of innate and adaptive immune response will be considered, and particular attention will be paid to suppression of the inflammatory reaction by nanoparticles (i.e., anti-inflammatory activity).

The explosion of research into engineered nanomaterials and nanomedicine has resulted in the discovery of unique immunological responses elicited by nanomaterials.

A pre-eminent goal of nanomedicine had always been to engineer nanomaterials that target specific tissue and deliver drugs, while simultaneously avoiding undesirable immunostimulatory or immunosuppressive reactions.[23–25] However, the inherent immunological response to nanomaterials may be also exploited for novel therapies. Immunological response can be modulated and suppressed through multiple pathways by the engineering the physiochemical properties of the nanomaterials. Between 2007 and 2010, Dobrovolskaia *et al*. produced comprehensive summaries of the basic structure–activity relationships of engineered nanoparticles and the immune system.[26–28] These reviews concluded that the immunological response to engineered nanomaterials can be attributed to the milieu of physiochemical properties, such as particle size, shape, charge, and surface chemistry.[23,29,30] For example, simple differences in nanoparticle size can alter cellular internalization, such that larger nanoparticles (>100 nm) are internalized and transported by macrophages and dendritic cells of the innate immune system, while smaller nanoparticles can easily travel to and accumulate in lymph nodes and effect B cells and T cells.[31,32] Modifying the surface of nanomaterials also alters the nanoparticle/biofluid interface which influences the way nanoparticles interact with and are labeled by plasma proteins. This effect is critical in determining the nanoparticles' interaction with cells, *in vivo* organ distribution and clearance pathways.[33–36]

While the fundamental structure–activity responses are still being investigated there is concurrent research into exploiting the immunological response to nanomaterials for novel direct immunostimulatory to immunosuppressive therapies.[30,37] Many researchers are investigating novel immunostimulants, such as nanoparticle-based or nanoparticle-supported vaccines.[28,38,39] Alternatively, the immunosuppressive capabilities of nanoparticles are garnering much attention. These research efforts involve understanding the direct interactions and effect of nanomaterials to suppress immunological signaling pathways. Direct immunosuppression can be applicable in overcoming autoimmune disease, reducing allergic reactions, and anti-rejection treatment. In the past year, Dobrovolskaia *et al*. also highlighted the burgeoning exploration of immunosuppressive and anti-inflammatory properties of nanomaterials.[37,40] Herein, we review recent reports on nanomaterials and methods to induce an immunosuppressive reaction through direct nanoparticle interactions with immune cells. We take in to account different nanoparticle chemistries and note the differences of immunosuppressive interactions with both innate immune cells (macrophages, dendritic cells, neutrophils, mast cells, and natural killer cell) and adaptive immune cells (T cells and B cells). We also discuss the effect of nanoparticles on the common immune reaction of inflammation.

Immunosuppressive nanomaterials

The size, shape, and chemistry of nanoparticles facilitate the binding of blood, cellular, and protein components which facilitate interactions with the immune cells and result in the immunological response. In general, the physiochemical properties of nanoparticles are important factors that significantly influence the interaction of nanoparticles and cells. Gold nanoparticles are particularly exemplary systems to illustrate these effects.[41,42] Spherical gold nanoparticles between 5 and 30 nm in diameter are capable of interacting with cells by passive means; however, larger nanoparticles and rod-like nanoparticles are more commonly internalized *via* complex uptake processes.[43–45] The surface-coating of the gold nanoparticles also effect cellular uptake. Where small-molecule organic ligands like citrate or lipids may promote stability and passive cellular uptake, macromolecular coatings like poly(ethylene glycol) may result in protein adsorption and reduction in cellular uptake.[44,46] Accordingly, the wide variation in size, shape, and surface coating of nanoparticles limit broad generalizations about the interactions of nanoparticles with the immune system. In the following discussion, we classify the nanoparticles into four groups based on chemical constituency: 1. metal nanoparticles, 2. metal-oxide nanoparticles, 3. carbon nanomaterials, and 4. polymer nanoparticles and macromolecules, and we described how they modulate and immunosuppressive response related to their physicochemical properties. Figure 1 illustrates the representative pathways nanoparticles may directly interact with the immune system and instigate an immunosuppressive response.

Metal nanoparticles

Metal and metal-oxide nanoparticles make up a significant share of engineered nanomaterials that are produced.[47] Accordingly, most efforts in to understanding the immunological response to nanomaterials have been related to occupational and environmental exposure to metal and metal-oxide nanoparticles.

Noble metal nanoparticles, such as gold and silver, interact with both the innate and adaptive immune systems,[48–51] but there are few reports that uncover the mechanism behind noble metal nanoparticles' ability to elicit an immunosuppressive response. Injection of organo-gold compounds has been utilized for nearly a century to treat inflammation, and only recent reports provide the first analysis of the biochemical pathway in which gold nanoparticles may reduce inflammation.[52,53] Citrate-coated gold nanoparticles were reported to not cause detectable cell or organ toxicity in mice, but the report concluded that citrate-coated gold nanoparticles showed anti-inflammatory activity and inhibited cellular responses induced by interleukin 1 beta (IL-1β). IL-1β is an inflammatory cytokine that acts as an arbiter between the innate and adaptive immune response; moreover, common inflammatory disorders, for example, rheumatoid arthritis, are mediated by IL-1β production. Monodispersed citrate-coated gold nanoparticles of different sizes between 5 and 35 nm were evaluated for their ability to modulate the pro-inflammatory function mediated by IL-1β production. The smallest nanoparticles, 5 nm, exhibited complete disruption of the IL-1β pathway. Larger nanoparticles, >10 nm, exhibited lesser effect, and 35 nm particles showed no effect on the IL-1β pathway.[45,54] The biochemical mechanism of the down-regulation of

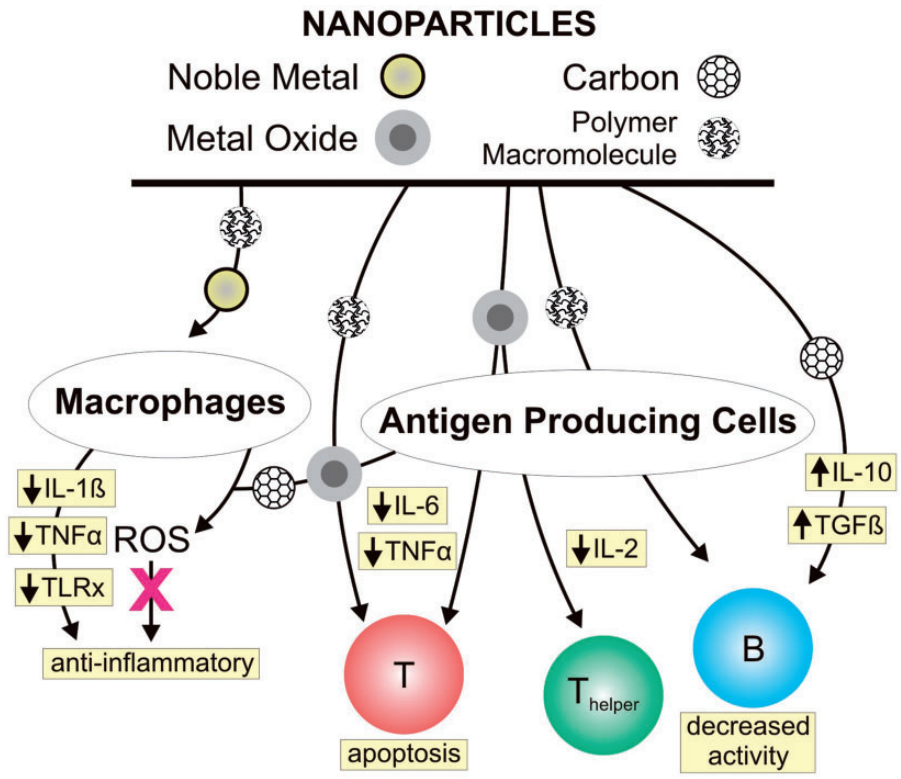

Figure 1 Direct interactions of nanoparticles with the immune system. Dependent on physiochemical properties, the nanoparticles interact with the constituents of the immune response, including macrophages, antigen presenting cells, B cells or T cells. Direct effects of carbon nanomaterials include the upregulation of transforming growth factor-β (TGFβ), interleukin-10 (IL-10), decreased B cell activity, as well as apoptosis. Metal-oxide nanoparticles can directly affect adaptive immune cells, and materials like cerium oxide nanoparticles scavenge reactive oxygen species (ROS) and operate as anti-inflammatory agents. Polymer nanoparticles and macromolecules (dendrimers) exhibit an array of immunosuppressive effects. The pathways shown are representative examples by which different nanoscale products might suppress the immune system. Immunosuppressive effects are modified from Smith *et al.*[30] and Jiao *et al.*[37] (A color version of this figure is available in the online journal.)

IL-1β induced inflammation pathways is assigned to extracellular interaction of citrate-coated gold nanoparticles with IL-1β. These results support prior research concluding that gold nanoparticles smaller than 10 nm exhibit unique interactions with cells.[55] Gold nanoparticles with diameter of approximately 200 nm and coated with poly(acrylic acid) showed the opposite response, as they promoted inflammation in the same THP-1 cell line.[56] These reports suggest the surface coating of the gold nanoparticles determine the cellular and immune system interaction that results from exposure to gold nanoparticles. The surface charge of stabilizing ligands also determine the immunological fate of the gold nanoparticles. Ultra-small zwitterion-stabilized gold nanoparticles (< 3 nm) were reported to directly instigate an immunosuppressive response without any measurable cytotoxicity.[57] Alternatively, other polymer and organic coatings did not produce comparable anti-inflammatory responses.[58–60] This suggests the size and coating either perform in unison or direct different pathways that induce the immunosuppressive response. Decoupling of these mechanisms would provide a clear direction to engineer better gold-based nanomedicines with the ability to regulate the IL-1β pathways for the treatment of chronic rheumatic disease.

Unlike gold nanoparticles, immunosuppression caused by silver nanoparticles has not been as widely reported. Silver nanoparticles have been reported to stimulate the production of cytokines, including TNF-α, IL-1, IL-6, IL-8, and IL-11.[61–63] Cytokines play an important role in wound inflammation, and Tian *et al*. reported the modulation of cytokines at a wound site by topical application of silver nanoparticles. Expression levels of IL-6 mRNA were significantly lower during the healing process while TGF-β1 levels were higher.[64] The reduction of inflammation with topical application[65] and systemic application[66] has also been reported. It is important to note, topical application of silver nanoparticles exhibited reduction of inflammatory cytokines, but also showed extensive apoptosis of inflammatory cells.[65] Continued exploration of the interaction of silver nanoparticles with the immune system will benefit our understanding in designing for the application of silver nanoparticles in fields such as medical devices, antimicrobial systems, and drug delivery.

Metal-oxide nanoparticles

Metal-oxide nanoparticles have been reported to induce both immunosuppressive and anti-inflammatory responses. Iron oxide nanoparticles have been shown to reduce the humoral immune response. The humoral response involves the recognition of antigens, allergens, pathogens, or foreign bodies in the blood with B cells.

In comparative reports by Liao *et al.* and Shen *et al.*, ovalbumin-sensitized mice were administered ovalbumin, a T cell-dependent antigen, following a dose of iron oxide nanoparticles. The production of antibodies specific to the antigen was significantly reduced along with spleen production of antigen-specific cytokines. Specifically, the iron oxide nanoparticle treatment blocked the activity of T_{helper} cells and macrophages and reduced the expression of interferon-γ, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α).[67,68] Both IL-6 and TNF-α are cytokines involved in systemic inflammation. The iron oxide nanoparticles also shifted the T_{helper} cell balance and suppressed the allergic reaction.[68] In an alternate mouse model, treatment with iron oxide particles prior to immune stimulation with an endotoxin reduced the production of IL-1β in microglia cells. The iron oxide nanoparticles inhibited pathways involved in cytokine processing which mediated the attenuation of IL-1β.[69] In a corollary report, human dendritic cells exhibited decreased antigen processing and decreased T cell stimulation when treated with poly(vinyl alcohol)-coated iron oxide nanoparticles. Additional injections of iron oxide nanoparticles resulted in a reduction of inflammation.[70] Iron-oxide nanoparticles have been reported to induce immunosuppression *via* multiple mechanism, as previously described; however, the review literature does not thoroughly differentiate the mechanism of action in which the iron-oxide nanoparticles interrupt or modulate the immune response. Jan *et al.* do report that the iron-oxide nanoparticles accumulated in lysosomes, which increased lysosomal permeability and decreased the activity of cathepsin B.[69] Cathepsin B is a secretory lysosomal enzyme involved in the activity of IL-1β, and its dysfunction may lead to the exhibited disruption of IL-1β production. A concerted effort to similarly explore and elucidate the fundamental interaction of iron-oxide nanoparticles with the biochemistry of immune cells or immune pathways would be beneficial to the current state-of-the art.

Metal-oxide nanoparticles also support an alternative anti-inflammatory response. Free-radical formation in the body has been attributed to inflammation, tissue damage, and development of diseases. Cerium can stably switch oxidation states between Ce^{4+} and Ce^{3+}, which enables cerium oxide nanoparticles to readily absorb free radicals. The oxidation and reduction of cerium oxide produces oxygen vacancies in the crystal lattice as it switches oxidation states. In nanoparticles, these vacancies are concentrated at the surface; therefore, cerium oxide nanoparticles have a unique capability to quickly react with free radicals. These antioxidant capabilities have been repeatedly demonstrated,[71] although the mechanism of their antioxidant capabilities and free radical scavenging in biological systems has not been isolated. Cerium oxide particles have been reported to mimic the superoxide dismutase active scavenging of superoxide (O_2^-).[72] Many other free radicals are in biological systems that can be implicated in the antioxidant and anti-inflammatory properties of cerium oxide nanoparticles. Of particular interest, cerium oxide nanoparticles scavenge highly reactive hydroxyl radicals.[73] In any case, the ability to scavenge reactive oxygen species can significantly reduce inflammation antagonized by reactive oxygen species.[74] Anti-oxidant properties of cerium oxide nanoparticles allow it to also inhibit inflammation generated from nitric oxide production by nitric oxide synthase.[75] Cerium oxide nanoparticles also caused the upregulation of superoxide dismutase-2, a mediator of oxidative stress.[76] Similar to many nanoparticles, antioxidant properties of cerium oxide nanoparticles depend on size and surface properties. As such, smaller cerium oxide nanoparticles possessed higher antioxidant capacity than their larger counterparts.[77]

Unlike cerium oxide nanoparticles, the single defining characteristics of the immunological response to zinc oxide nanoparticles is cytotoxicity related to oxidative stress from the increased production of reactive oxygen species.[78–80] Kim *et al.* explored the effect of surface charge and size on the immunological response to zinc oxide nanoparticles, and they concluded positively charged zinc oxide nanoparticles exerted higher cytotoxicity than the negatively charged zinc oxide nanoparticles. Further evaluation showed natural killer cell activity was suppressed; the $CD4^+/CD8^+$ T cell ratio (a measure of the ratio of T_{helper} to cytotoxic T cells) was slightly reduced, nitric oxide production from splenocyte culture was lower; and IL-1β, TNF-α, IL-10, and interferon-γ in zinc oxide were significantly suppressed. Each are cytokines related to pro-inflammatory response. Collectively, these responses suggest the charged surface of zinc nanoparticles elicit both dramatic and systemic immunosuppression, which correlated to the observed cytotoxicity in the same cells. The effect of nanoparticle size on immune cell cytotoxicity was evaluated, and similar to prior efforts,[81–83] it was reported that smaller particles exhibited a slightly higher EC_{50} than smaller zinc oxide nanoparticles The size of the nanoparticle also correlated to cytokine concentration in the serum; the serum levels of pro-inflammatory cytokines were reduced with increased particle size.[84]

Another common metal-oxide nanoparticle, titanium oxide, are constituents of many cosmetic and commercial colorants, included in products such as paints and plastics. Unlike zinc oxide nanoparticles, titanium oxide nanoparticles have been reported as non-cytotoxic *in vitro*,[85,86] but they can induce an immunosuppressive response. In a tumor model, titanium oxide nanoparticles inhibited T-cells, B-cells, macrophages, and natural killer cells.[87]

Carbon nanomaterials

Carbon nanomaterials exhibit strong free-radical scavenger properties and can be utilized as anti-inflammatory agents.[88–90] Similar to cerium oxide nanoparticles, fullerenes (C_{60}) decrease the level of reactive oxygen species by efficiently scavenging free-radicals. Unlike cerium oxide nanoparticles, fullerenes distribute the free-radicals through their aromatic structure.[91] Fullerenes reduce reactive oxygen species of both hydroxyl and superoxide radicals, and many of the radical scavenging properties are moderated by the functionalization of fullerenes with water-soluble ligands.[71,92] Fullerenes have been shown to suppress oxidative stress *in vitro* and *in vivo* models, but immunological response is highly dependent on dosing and delivery.[93] Both intraperitoneal injection and inhalation

delivery of low doses of fullerenes exhibited reduction in oxidative stress. Hydroxylated fullerenes were used to protect against oxidative stress in RAW 264.6 cells *in vitro* and ischemia-perfused lungs in rats.[94] Reduction in signaling pathways associated with reactive oxygen species levels also enable fullerenes to inhibit human mast cell and peripheral blood basophil release of meditators. Mast cells and peripheral blood basophils are cells involved in the initiation of inflammatory response and Type I hypersensitivity allergies. Type I hypersensitivity allergies are the result of repeated exposure to an allergen. After initial exposure, B cells produce the allergen-specific Immunoglobulin E (IgE) which then binds the receptors and sensitizes mast cells and peripheral blood basophils. Subsequent exposure to the allergen causes crosslinking of the IgE and activates mediators such as histamine and prostaglandins. After treatment with fullerenes, the mast cells and peripheral blood basophils decreased this IgE signaling and decreased ROS production, which prevented histamine release.[89] Many allergy medications are aimed at a similar response for neutralizing the IgE response. Studies examining the toxicity of fullerenes on biological systems are still ongoing, and an *in vivo* evaluation of the immunological response to fullerenes may still pave the way for other treatments of autoimmune disorders.

Other allotropes of carbon nanomaterials exhibit a less clear mechanism for immunosuppressive action. Mitchell *et al.* have reported the inhalation of multi-walled carbon nanotubes at low concentrations in mouse models lead to stimulation of pathways responsible for T-cell dysfunction and suppressed immune response from spleen cells; however, they report no cytotoxicity or immunosuppression in the lungs. Exposure to the multi-walled carbon nanotubes also resulted in decreased natural killer cell function, increased production of prostaglandin and increased IL-10.[95,96] The signaling and transference of immunological response from the exposed lungs to the spleen is very interesting. For comparison, single-walled carbon nanotubes were reported to suppress immune system mediators in human lung epithelial cells.[97] Other studies showed that inhalation of single-wall carbon nanotubes in mouse models induced pulmonary inflammation and suppressed the responsiveness of T cells after exposure; this immunosuppression was associated with the direct effects of single-wall carbon nanotubes on dendritic cells.[98]

The reported differences in immunological response between fullerenes, multi-wall and single-wall carbon nanotubes may be attributed to their geometries as well as their different electrochemical properties. Future efforts to decouple these effects may lead to a better understanding of surface charge and inherent conductivity on immunological response.

Polymer nanoparticles, macromolecules, and liposomes

A popular alternative to inorganic and carbon nanomaterials are polymer nanoparticles and macromolecules. A majority of polymer nanoparticles and macromolecules have been utilized as carriers of drugs for indirect immunosuppression. Polymer and other macromolecular nanomaterials are also very popular for induction of immunostimulatory response. There have been a few nanotoxicology studies to explore the immunological response to simple polymer nanomaterials. One particular class of polymer nanoparticles to be examined for immunological response is polystyrene latexes. Polystyrene latexes are very common industrial polymers that provide a simple model to study the bio-activity of nanoparticles dependence on size and surface chemistry. Unfortunately, the tunability of polystyrene latexes are their only simple characteristic. Polystyrene latexes have been reported to induce both immunostimulatory and immunosuppressive responses. During an allergen challenge, polystyrene nanoparticles inhibited lung inflammation and this reaction was attributed to the inhibited expansion of dendritic cells in the lungs.[99] Another study with polystyrene nanoparticles emphasized the importance of surface charge on immunological response. Frick *et al.* altered the surface charge of polystyrene nanoparticles by decorating them with charged sulfonate and phosphonate groups. This resulted in immunostimulation by dendritic cell maturation and enhanced $CD4^+$ T cell activity.[100] Finally, antigen-decorated polystyrene nanoparticles also induced T-cell tolerance and suppressed autoimmune encephalomyelitis by inactivating pathogenic T cells.[101] The significant impact of surface groups on the immunological response to simple polystyrene nanoparticles illustrates the necessity for continued research into the immunology of polymer nanoparticles that are so often praised and developed for drug deliver and other nanomedicine applications.

Polymer macromolecules, specifically dendrimers, have been explored as potential anti-inflammatory agents.[102] Anti-inflammatory properties of polyamidoamine dendrimers were unexpectedly discovered while investigating their application for a drug delivery system. Polyamidoamine dendrimers possessing amine or hydroxyl surface groups substantially reduced pro-inflammatory responses. Dendrimers presenting carboxylate surface groups did not show any enhanced anti-inflammatory properties.[103] Hydroxyl-terminus polyamidoamine dendrimers also blocked the release of pro-inflammatory regulators nitric oxide and IL-6 in microglia cells.[104] Hayder *et al.* also modified the surface charge of dendrimers with anionic azabisphosphonate group. Arthritic mouse models were treated with these anionic dendrimers and they inhibited the secretion of pro-inflammatory cytokines and stunted the osteoclastogenesis process.[105]

Dendrimers also enable a unique hybrid of direct and indirect immunosuppression. As previously mentioned, indirect immunosuppression involves the delivery of an immunosuppressant, for example, gluco-steroid or cyclosporine A, with a nanoparticle as the delivery vehicle. Dendrimers have the unique capability to be both the delivery vehicle and payload, since they can be composed of repeating small-molecule or incorporate immunosuppressive molecules within their macrostructure. For example, carboxylic acid terminated polyamidoamine dendrimers did not significantly reduce inflammatory response; however, when these dendrimers were conjugated with

glucosamine, dendritic cell, and macrophage activity was inhibited.[106,107] The polyamidoamine-glucosamine dendrimers inhibit toll-like receptor (TLR) mediated inflammatory responses, and they have been shown to decrease IL-6 and IL-8 production.[108] TLR mediate the innate immune system response, and they are expressed in macrophages and dendritic cells. Analogous to the polyamidoamine-glucosamine dendrimers are imidazoquinoline-based dendrimers, which are dendrimers with the base constituent being imidazoquinoline instead of glucosamine. Imidazoquinoline is an agonist for TLR7 and TLR8; thus, the imidazoquinoline-based dendrimers suppress both TLR7 and TLR8 activity.[109] Although the mechanisms by which particular pathways are suppressed are not fully understood, it is clear that the surface charge and increased chemical density of the dendrimers play a very impactful role. This highlights how simple surface charge and surface chemistry manipulation at the nanoscale level can be used to engineer nanoparticles that finely tune the innate immunological response.

Liposomes are another class engineered nanoparticles; liposomes are lipid bi-layer spheres. Liposomes can be used to enhance localized delivery of encapsulated immunosuppressive agents. Hong *et al*. evaluated the efficacy of delivering an IL-10 gene within cationic liposomes to increase allograft survival following a heart transplant. Delivery using liposomes resulted in local overexpression of the IL-10 and a reduction in lymphocyte responsiveness.[110] Similarly, canines were administered liposomal tacrolimus, an immunosuppressive drug, following a liver transplant and survived significantly longer than canines who were given tacrolimus intravenously.[111] Glucocorticoids encapsulated within liposomes provided for dose reduction when applied to rheumatoid arthritis in rats, resulting in reduced toxicity and increased suppression of anti-inflammatory cytokines when compared to administration of free drug.[112] As this illustrates, liposomes have been utilized as a carrier for multiple immunosuppressive therapies; however, a more probing evaluation of the direct interaction of ''cargo-free'' liposomes with the immune system after systemic or environmental exposure will be of particular interest in the future.

Similar to liposomes, peptide and peptide-based amphiphiles are macromolecules which self-assemble into either nanoparticles or nanofibers *in situ*, and current research efforts are focused on utilizing these nanomaterials for delivery of immunotherapeutics. As described by Tirrell *et al*., a majority of peptide amphiphile delivery systems are developed to induce immunostimulatory effects in the innate and adaptive immune system.[113–115] Although these reports describe the capability of peptide amphiphile micelles to cause immunostimulation, based on their direct interaction with the immune system, there may be future potential to use similar nanomaterials generate immunosuppressive responses.

As demonstrated by the myriad macromolecular nanoparticles discussed heretofore, there is significant potential to decorate and functionalize macromolecular nanoparticles to modulate the immune response. Similar to metal and metal-oxide nanoparticles, the surface chemistry of macromolecular nanoparticles direct cellular targeting, uptake, and bioactivity. Accordingly, researchers are exploring nanoparticle chemistries and coatings to modulate immune responses.

Complications with nanoparticle immunosuppresion

In the best circumstances, immunosuppressive therapies would be targeted to hyperactive components of the immune system in the case of an autoimmune disorder, or the immunosuppressive therapy could be acutely active to coincide with pharmaceutical treatment and then immediately dissipate. In both scenarios, the immunosuppressive therapy and would not result in any immunodeficiency. Nonetheless, current immunosuppressive therapies have the potential to cause immunodeficiency which results in increased susceptibility to opportunistic pathogens, degradation of bone marrow (myelosuppression), and increased cytotoxicity and genotoxicity to the active nanomaterial.

Since small changes in nanoparticle size, shape, charge, and constituency result in magnified immunological response, it will be necessary to continue expansive research into the toxicology and immunology of nanoparticles. In 2009, Bregoli *et al.* reported a toxicity analysis of different metal and metal-oxide nanoparticles that highlights the dramatic variability of immunological response to nanoparticles, and frames the current viability of nanoparticles as immunomodulators. Variants of iron oxide, antimony oxide, gold, titanium oxide, and cobalt and silver nanoparticles were introduced to hematopoietic progenitor cells. Hematopoietic progenitor cells are bone marrow derived cells that give rise to myeloid and lymphoid lineages of immune cells. Only antimony oxide and cobalt nanoparticles exhibited a toxic effect. Antimony oxide nanoparticles were specifically toxic to erythroid progenitors. Cobalt nanoparticles were toxic to both erythroid and granulocytic-monocytic progenitors. On the other hand, in a complimentary assay in which antimony oxide nanoparticles were tested against immortalized cell lines of hematopoietic origins, there was no displayed toxicity.[116] Different types of immune cells and their lineage may exhibit different sensitivities to the same nanoparticle type. Analysis of the immunological response of zinc oxide nanoparticles has resulted in similarly complex results; reports have described immunosuppression, immunostimulation, and general cytotoxicity of zinc oxide nanoparticles.[84,117] Zinc oxide nanoparticles induced high levels of toxicity and increased ROS production in monocytes, while lymphocytes remained relatively resistant to the toxic effects.[80] These contradictory results are unfortunately the hallmark of nanotoxicology research and it raises questions on the utility and methods of analyzing immunological response to nanoparticles with *in vitro* cell cultures.

As the factors effecting immunological response to nanoparticles are further investigated at the fundamental level, it should be noted that in application, these factors may exhibit a compounding effect. For example, if the nanoparticle delivery vehicle for an immunosuppressant or

chemotherapeutic creates its own immunosuppressive response, the drug's activity may be exponentially compounded. It has been reported that doxorubicin bound to polymer nanoparticles both stimulated greater myelosuppresive affects than doxorubicin alone, and the nanoparticles released stimulating factors they were attributed to an increased toxicity of doxorubicin.[118] This can cause potential problems with nanoparticles due to their ability to deliver precise and localized drug dosing.[119,120] It should be considered that the nanoparticle formulation of a drug may not only increase the efficacy of the drug by precise delivery and increased circulation time, but there may be a synergistic immunological response caused by the nanoparticle.

Outlook

Heretofore, we have discussed direct immunosuppression induced by nanoparticles. There is a great potential to expand our understanding of immunosuppression by nanoparticles, and further studies need to be conducted to completely evaluate fundamental mechanisms in more relevant models. These efforts will assist in understanding why some nanoparticle complexes are immuno-stimulatory *in vivo* while immunosuppressive *in vitro* or other combinations thereof.

Ultimately, associations of pathway regulation with nanomaterials size, structure, and method of introduction to tissue needs to be comprehensively evaluated in order to introduce these nanomaterial applications clinically.[9,121,122] While immunological reaction to nanoparticles are being intensely investigated, their direct immunosuppressive and anti-inflammatory properties may hold the necessary keys to the next generation drug delivery vehicles and complimentary treatments for regenerative medicine and transplantation. The cooperative research among materials scientists, immunologists, and toxicologists is a very exciting field expected to progress alongside the development of new nanomedicines.

ACKNOWLEDGMENTS

All authors participated in the research and writing of the manuscript. The authors are funded by North Carolina State University. Funders had no role in the preparation of the review or the decision to submit for publication.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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