Advanced Review

# Interactions of nanomaterials with the immune system

Salik Hussain,[1,2†] Jeroen A. J. Vanoirbeek[2] and Peter H. M. Hoet[2*]

Evaluation of the immunomodulatory potentials of nanomaterials is essential for developing safe and consumer-friendly nanotechnology. Various nanomaterials interact with the immune system, in a beneficial or deleterious way, but mechanistic details about such interactions are scarce. A lack of agreed-upon guidelines for evaluating the immunotoxicity of nanoparticles (NPs) adds to the complexity of the issue. Various review articles have summarized the immune system interactions of biodegradable NPs (with pharmaceutical uses), but such information is largely lacking for nonbiodegradable NPs. Here we give an overview of interactions of nonbiodegradable, persistent NPs with the immune system. Particular emphases include key factors that shape such interactions, cell-specific responses, allergy and immune-sensitive respiratory disorders. 

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## INTRODUCTION

The exploration of the immunomodulating potential of nanoparticles (NPs), encompassing both immunostimulatory and immunosuppressive components, has only recently been a focus of research into the health effects of nanomaterials. However, little data are available on the mechanisms involved in such effects.

An in-depth evaluation of such effects with both accidental exposure (e.g., environmental and occupational) and therapeutic exposure (vaccinations, drug delivery tools) is required. For both voluntary and involuntary exposure, knowledge is limited, and questions such as how NPs can interact with the immune system and which effects are expected in both the short- and long-term remain unanswered. Novel immunotoxicological assessments of nanomaterials were previously suggested to need stepwise validation, standardization, and demonstration of physiological relevance[1] (Box 1, Figure 1).

### BOX 1

#### IMMUNOTOXICOLOGY

Immunotoxicity can be defined as any adverse effect on the immune system that can result from exposure (Figure 1). To determine the effects of an agent on immune function, in general five adverse event categories are studied.

1. Immunosuppression refers to impairment of any component of the immune system resulting in a decreased immune function.[2]

   The impairment can be observed as myelosuppression, changes in immune system organ weights, and decreased serum globulin levels. Functionally, it can result in an increased incidence of infections and/or tumors.
2. Immunogenicity is immune reactions elicited by a specific stressor (agent) and/or its metabolites, possibly resulting in an allergic response, on multiple exposure to the stressor.
3. Hypersensitivity is the immunological sensitization by a specific stressor (agent) and/or its metabolites, resulting in a strong adverse response; four types of hypersensitivity responses have been described.[3]

†Present address: National Institute of Environmental Health Sciences, National Institute of Health, Research Triangle Park, Durham, NC, USA

*Correspondence to: peter.hoet@med.kuleuven.be

[1]Unit of Functional and Adaptive Biology, Laboratory of Molecular and Cellular Responses to Xenobiotics, Université Paris Diderot, Paris, France

[2]Research Unit for Lung Toxicology, K.U. Leuven, Leuven, Belgium

  

4. Autoimmunity refers to a pathological process whereby the immune system responds to self-antigens.
5. Adverse immunostimulation refers to any antigen-nonspecific, inappropriate, or uncontrolled activation of some component of the immune system, including activation of immune-system effector mechanisms.

(Abstracted from Refs 4 and 5).

Initial recognition of nanomaterials by the immune system is an essential determinant for the fate and distribution of these materials inside the body. Moreover, functional impairments incited by these nanomaterials to the immune system, such as engulfment of cellular debris by macrophages and antigen presentation to T lymphocytes, also affect the final outcome after exposure to the nanomaterials.

Depending on persistence in the body, nanomaterials can be broadly described as biodegradable or nonbiodegradable. Biodegradable NPs have various medicinal/consumer product applications and have been the subject of various publications dealing with their possible interactions with the immune system.[1,6] Nonbiodegradable NPs have important industrial applications and can be important from an environmental point of view, but much less is known about their interactions with the immune system. This is not a stringent criterion for the classification of NPs and exceptions do exist (e.g., utilization of gold (Au) in nanomedicine sector). In this review, we focus on modulation of the immune response after exposure to biopersistent nanomaterials, with emphasis on key factors that participate in the final outcome of the immune response. Moreover, we discuss the modulation of immune-sensitive respiratory disorders by NPs.

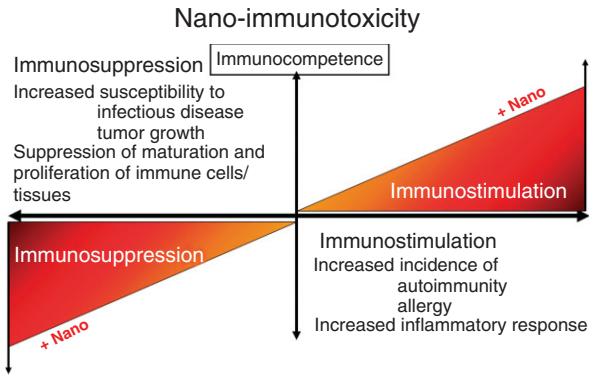


**FIGURE 1** | Schematic overview of nano-immunotoxicology. Immune dysfunction resulting from exposure to nanomaterials/particles may take the form of a specific stimulation (of one or different compartments of the immune system) leading to, for example, allergy, autoimmunity etc. or it may take the form of an immunosuppression leading to more infectious pathologies and tumor growth.

## Immune System Components and Their Specific Modulation by NPs

The primary function of the immune system is to prevent or protect against foreign material, mostly micro-organisms, but also dust and particles, entering and/or affecting the organism. In the defense against foreign intruders, several lines of protection, both specific and nonspecific, are integrated. These lines of defense are divided into the innate and adaptive immune response (Box 2).

### BOX 2

#### INNATE AND ACQUIRED IMMUNITY

The innate immune system is our first line of defence against invading organisms and can immediately respond to any stressor. It consists of four different protective barriers: (1) anatomic or physical, blocking the material from entering the organism (skin/surface of mucous membranes); (2) physiologic, increasing blood flow, degradation of material, and activation of the immune system (by temperature, pH, oxygen tension, or soluble factors such as lysozymes, interferon, and complement); (3) endocytic and/or phagocytic, active uptake of the material by specialized cells; and (4) inflammatory, recruitment of different cells (mainly macrophages and neutrophils). The innate immune system is nonspecific and has no memory (will respond each time—during reexposure—in a similar way).

The adaptive immune system acts as a second line of defence and can respond efficiently to reexposure to the same pathogen. The adaptive immune system may take days to respond to a primary exposure. In the adaptive immune system, antibodies (soluble proteins that bind specifically to antigens) are produced, as are cell-mediated responses in which specific cells recognize foreign pathogens and destroy them. The response to a reexposure is rapid because low titers of specific antigens are already present and memory B and T cells are activated.

The anatomic barriers such as the skin form an important first line of defence, but once this hurdle is overcome, the foreign material faces phagocytic cells.

These cells can activate the three other innate barriers. The macrophage is the most abundant or known phagocytotic cell and appears in different critical tissues in different forms (e.g., Kuppfer cells in the liver).

Many phagocytic cells such as dendritic cells (DCs) and macrophages can present specific antigens [also known as antigen-presenting cells (APCs)] and thereby form the connection between innate and acquired (adaptive) immunity, for example, by secreting chemotactic peptides that recruit lymphocytes or by presenting specific antigens to specialised cells. The main feature of the adaptive immune system is its specific, inducible response. Phagocytic cells are general-purpose effector cells capable of handling a wide variety of stressors, whereas lymphocytes (acquired immunity) are specific to a single stressor.

Both systems (innate and acquired) strongly interact, and drawing a line between these systems is often impossible. At first contact, cells of the immune system interact with the foreign materials, through direct interaction or with the help of APCs. This process is accompanied by the production of different cytokines, which act as inflammatory and/or immunological mediators. These cytokines or chemokines can shape the immune response toward a pro-inflammatory or an anti-inflammatory outcome.

## INNATE IMMUNE RESPONSE

As soon as nanomaterials invade an organism, they interact with biological molecules, most often proteins such as albumin but also members of the complement system. These interactions shape the material-induced biological effects.

Here, we focus on reports describing the responses of different immune cells, particularly in humans, after exposure to NPs and the resulting outcome in terms of cytokine production and/or cellular responses. Various studies have focused on one or more critical functions of macrophages in the inflammatory response, which include phagocytosis and production of inflammatory mediators (cytokines).

### Barrier Function

The skin, intestinal tract, and lungs are in direct contact with the environment and can therefore be directly exposed to nanomaterials. The skin acts primarily as a barrier, but lungs and the intestinal tract also allow transport (passive and/or active) of substances such as oxygen, water, and/or nutrients. These sites of exposure are also the main entry site for nanomaterials. In the body, after entering via one of the portals or after injection, NPs can translocate through diverse epithelial/endothelial barriers.[7–9]

Skin is an important barrier to exogenous agents and an essential part of the early immune response with dermal exposure to NPs. For directly interacting with immune cells in skin, NPs need to penetrate to deeper skin layers. The transcutaneous penetration of titanium dioxide ($TiO_2$) and zinc oxide (ZnO) NPs is particularly studied for applications in sunscreens and cosmetics, whereas silver (Ag) NPs are studied as antibacterial coatings for clothes.[10] Since the production of NPs has blossomed worldwide, occupational skin exposure to specific materials, such as the fullerene aggregate C60, has become a research topic. C60 penetrates the primary skin barrier, but no data exists on the transport of C60 in the viable epidermis or dermis.[11]

The healthy skin seems to be an efficient barrier to most NPs, but damaged skin (e.g., by wounds, erythema, eczema) and flexure sites are more susceptible to NP translocation. In vitro experiments with human skin submitted to mechanical flexions (20 flexions of 45° per min) have shown that in half of the samples, flexions favor a low epidermal and dermal absorption of fluorescent NPs (0.5 and 1 μm) after a 60-min exposure, whereas particles of larger size (2 and 4 μm) stay localized on the strateum corneum.[12] For systemic passage, NPs must cross the epidermis and the basal membrane. Some evidence suggests that skin is permeable to nanomaterials with specific physicochemical properties.[13] Various recent reviews have focused on the skin penetration of NPs and highlighted the discrepancies in the literature about their translocation to deeper viable skin.[14–16]

Exposure to NPs orally can lead to translocation across the gastrointestinal tract through tight junctions (paracellular), DCs, and transcytosis through microfold cells.[17] Several studies investigated nanocarriers to increase the bioavailability of drugs. These carriers (e.g., liposomes and dendrimers) are of great interest but are designed not to be biopersistent.[18]

The inhalation or instillation of NPs has been examined in numerous studies and found to often induce local inflammatory effects in the lung. NPs can translocate from the lung to the circulation and reach secondary target organs.[9] However, the mechanism(s) involved are not fully understood.[19–24]

### Effect of NP-adsorbed Proteins (Peptides) on the Immune Response

The biological fate and (re)biodistribution of NPs strongly depends on the physicochemical characteristics of the particles and the proteins that NPs

  

encounter in the body, particularly in plasma.[25–28] A number of serum proteins have recently been identified to bind to carbon black (CB), $TiO_2$ or acrylamide NPs.[25,29,30] Among the proteins identified, several, such as apoliprotein E, granulocyte macrophage colony-stimulating factor (GM-CSF) or transferrin, are ligands for cellular receptors. Although the structural and functional status of these proteins absorbed on the NP surfaces have not been addressed in these studies, these proteins may contribute to the biological effects of NPs through activation/inactivation of receptor-dependent signaling.[28]

The amount, along with the structural and functional properties of the adsorbed proteins, shapes the interactions of these nanomaterials with the cells and contributes to their biological responses.[25] Moreover, such interactions depend on the chemical nature, surface, and size of the NPs. Further in-depth evaluation of such interactions can help in understanding the nano-biointeractions and toxicological events arising from such interactions. Another important consideration is the possibility of conformational changes in the structure of adsorbed proteins. Such changes have been shown for a few NP types (e.g., Si NPs induced a helical structure, including a catalytic site, on unstructured peptides in solution[31]). Under in vivo conditions, such interactions may lead to a change or loss of function of the adsorbed proteins and may also result in presentation of novel peptide motifs to the immune system. In a recent publication, the authors speculated that such interactions can also lead to autoreactivity against self-epitopes and may result in a persistent cell-mediated immune response,[32] but further mechanistic studies are needed to confirm such hypotheses. The proteins adsorbed from blood to the NP surface are mainly immunoglobulins and components of the complement system, which may act as signals for innate and/or adaptive immune responses.[26,33–35] Purified single-wall carbon nanotubes (SWCNTs) and double-wall CNTs (DWCNTs) have been shown to activate the human serum complement system in a potent manner (comparable to equal weight of zymosan) by the classical pathway of human serum complement activation.[36] Moreover, DWCNTs can also activate the alternate pathway of complement activation. This activation of complement was due to selective binding of C1q CNTs (classical pathway activation), whereas C3b binding was postulated as the mechanism of alternate pathway activation.

The phenomenon of protein binding is important in immune responses, but how to study it is unclear. A fundamental obstacle is the observation that the nature of adsorbed proteins on the NPs also depends on the cell culture media. Maiorano et al. recently showed that the nature of proteins adsorbed on Au NPs differed depending on use of Dulbecco modified Eagle's medium or Roswell Park Memorial Institute medium to culture cells and influenced the internalization pattern or amount of NPs.[37] These results demonstrate the significance of properly characterizing all constituents before experimentation.

## Inflammation and Phagocytosis

Inflammation is an integral part of the immune response. The inflammatory or in vitro pro-inflammatory effects of NPs are one of the most widely studied phenomena in nanotoxicological studies. However, most of these reports mainly described changes in a few cytokines or chemokines but lacked mechanistic data. Moreover, NPs can reach distinct body organs after translocating through diverse epithelial or endothelial barriers, which gives particular significance to the evaluation of their interaction with different types of macrophages and other immune cells. Particular attention must be paid to evaluating the inflammatory potentials of NPs because various types of NPs (e.g., CB and $TiO_2$) can adsorb pro-inflammatory mediators [refer to *Effect of NP-adsorbed proteins (peptides) on the immune response* section]. The adsorption of GM-CSF, interleukin 6 (IL-6), and tumor necrosis factor $\alpha$ (TNF-$\alpha$) on CB (13 nm) and $TiO_2$ (15 nm) NPs under in vitro conditions has been confirmed.[30,38]

Here we describe the few selected, recent publications on the acute effects (in vitro and ex vivo) of nanomaterials on macrophages, derived in vitro from activated monocyte cell lines or freshly isolated after in vivo exposure, DCs, neutrophils and mast cells.

### *Macrophages*

Human macrophages [cell line U937 maturated with phorbol myristate acetate (PMA)] were used in a study by Lucarelli et al. to compare the immune toxicity of ceramic oxides such as Ti (70 nm), Si (15 nm), and Zr (5–30 nm) with metallic Co NPs (50–200 nm).[39] Cells exposed to the highest nontoxic doses of NPs (determined by XTT assay) showed changed expression of toll-like receptor (TLR) and pro-inflammatory cytokine production. From expression studies of TLRs, the authors suggested the possibility of inadequate defense against certain infections after exposure to Ti and Si NPs (finding of inhibition of TLR9). Moreover, the authors suggested an increase/amplification of chronic inflammation with exposure to Zr NPs (increased expression of TLR7 and induction of TLR3) and a bias in naive macrophages

  

toward an inflammatory M1 functional phenotype after Si NP exposure. However, this study lacked adequate material characterization, and these results need to be confirmed under in vivo conditions. Wan et al. studied low-dose exposure (2.5 and 5 μg/mL) of 20 nm Co and $TiO_2$ NPs on the expression of matrix metalloproteinase 2 (MMP-2) and MMP-9 in monocytes/macrophages; only nano-Co caused an imbalance between the expression and activity of MMPs and their inhibitors. Additionally, activator protein-1 (AP-1) and protein tyrosine kinases (PTK) signaling pathways contributed to such effects.[40]

In the lung, alveolar macrophages are the principal cell type responsible for pulmonary immune and inflammatory response to inhaled particles. Ma et al. exposed rats to 20 nm $CeO_2$ NPs (up to 7 mg/kg body weight) through a single-instillation procedure and evaluated inflammatory and alveolar macrophage function changes.[41] Alveolar macrophages from $CeO_2$-exposed rats showed decreased nitric oxide (NO) production and increased IL-12 production in response to ex vivo lipopolysaccharide (LPS) exposure after 1 day. The authors also observed inflammation, cytotoxicity (toward alveolar macrophages), phospholipidosis, and enlargement of alveolar macrophages. Another interesting finding was a later (28 days' postexposure) increase in the mRNA expression of arginase-1, which is believed to indicate the switching of alveolar macrophages from an inflammatory (M-1) subset to a fibrogenic (M-2) subset. Liu et al. studied the effects of 5 nm $TiO_2$ NP instillation on rat pulmonary alveolar macrophages and observed that high-dose exposure resulted in suppressed phagocytic activity, decreased chemotactic ability, decreased expression of both Fc receptors and major histocompatibility complex (MHC) class II molecules on the macrophage surface.[42] The authors believed that the mechanism behind these effects increased NO and TNF-$\alpha$ production. Moreover, they observed significant particle size dependence (5 versus 200 nm) of these effects, with smaller NPs more effective than larger particles.

In coexposure models of NPs and infectious agents, the role of phagocytic macrophages was clearly hampered. Braydich-Stolle et al. studied the effects of Al and aluminum oxide ($Al_2O_3$) NPs in a coculture model (U937 and A549 cells) with or without prestimulation with a respiratory pathogen (*Staphyloccus aureus*; ca-MRSA) and found that NPs (25 μg/mL) inhibited the NF$\kappa$B activation induced by the respiratory pathogen. The inhibition of NF$\kappa$B abolished or even downregulated the production of different pro-inflammatory cytokines/chemokines such as IL-6, IL-1$\beta$, TNF-$\alpha$, and IL-8 or further upregulated the abolishing effect on IL-10.[43] These results are important because the NF$\kappa$B pathway is one of the key regulators of both the innate and adaptive immune response. Furthermore, the authors demonstrated that the presence of macrophages led to decreased cytotoxicity of epithelial cells. However, the mechanism of these protective effects was not elaborated. This study demonstrates that the ability of mounting an efficient immune response to foreign pathogens may be altered in the presence of NPs. However, these results need to be confirmed in vivo.

Shvedova et al. found that preexposure to SWCNTs (3–5 nm diameter, 1–3 μm long, and 10/40 μg/mouse) resulted in lowered lung clearance of *Listeria monocytogenase* in C57BL/6 mice.[44] This lowered clearance of bacteria was associated with impaired respiratory function. The authors speculated that this enhanced inflammatory response accompanied by delayed bacterial clearance could lead to increased susceptibility to lung infection in exposed individuals. In another in vitro study, primary human monocyte-derived macrophages exposed to 0.1 mg/mL purified SWCNTs (500 nm–2 μm long and 1–4 nm diameter) showed decreased ability to engulf apoptotic cells.[45] Moreover, the authors observed suppressed chemotaxis of primary human monocytes toward a standard chemoattractant [monocyte chemoattractant protein 1 (MCP-1)].

### *Dendritic Cells*

DCs are of prime importance in the immune response. They contribute to the coordination of innate and adaptive immunity and act as inducers of the adaptive immune response. An interesting study showed that polystyrene NPs interact with DCs in a size-dependent manner[46]: striking differences were shown between 20- and 500-nm NPs in terms of their transport through lymphatics, localization within the lymph nodes and association with DCs after footpad injections in C57BL/6 mice. Larger NPs were transported through the DCs (no transport seen in DC-depleted animals), whereas 20-nm NPs showed free transport and was taken up by lymph node-resident cells such as CD8a+, CD8a–DC, and plasmacytoid DCs. In another study, a 5% suspension of carbon-coated iron-oxide NPs of different sizes (10–200 nm) were incorporated into human DCs (matured from peripheral blood) in a size-independent manner and were localized within lysosomes.[47] The authors did not observe any cytotoxicity of these NPs toward DCs and proposed that the loading of DCs with these NPs could be a helpful strategy to improve cancer diagnosis and therapy. In the same line, Marcos-Campos et al. exposed magnetic NPs (the same as in the previous

  

study) loaded with DCs to alternating magnetic fields and observed a decrease in viability of DCs from 90 to 2–5%.[48] The authors discussed the possibilities of using magnetic NP-loaded DCs as a potentially powerful 'Trojan horse' system for NP delivery to specifically targeted sites. More recently, Kunzmann et al. studied the role of surface functionalization (Si versus dextran coating) and size (30 versus 50 nm) of iron oxide NPs on cytotoxic and inflammatory responses of primary monocyte-derived DCs.[49] The authors observed that Si-coated iron oxide NPs induced a dose-dependent toxicity, but the same-size dextran-coated iron oxide NPs were noncytotoxic. In this study, exposure to both types of NPs produced no pro-inflammatory cytokine secretion. The Si-coated iron oxide NPs were taken up to a significantly higher degree than the dextran-coated NPs, regardless of size. A recent study by Winter et al. showed the ability of Si (14 nm) and P25 $TiO_2$ NPs to activate murine bone marrow-derived DCs (CD11c and MHCII expressions), as well as activate inflammasome.[50]

### *Neutrophils*

Neutrophils are an essential component of the innate immune response and are effective defense mechanisms against bacteria and fungi. Although various types of NPs can lead to neutrophilic inflammation in the lungs, little is known about the effects of NPs on neutrophil physiology. Recently, the human neutrophil-agonist properties of $TiO_2$ NPs under in vitro conditions were demonstrated, with a prominent role for IL-8, the main neutrophil chemokine in humans[51]: as low as 0.002 μg/mL $TiO_2$ NPs induce rapid tyrosine phosphorylation events with an early and prominent role of extracellular signal-regulated kinases 1/2, p38 mitogen-activated protein kinase and IL-8. Bartneck et al. demonstrated that extracellular traps formed by myeloid immune cells (neutrophils, monocytes, and macrophages) act as physical barriers for NPs, which demonstrated a new nanomaterial clearance mechanism for the human immune system. More specifically, positive charges significantly enhanced particle clearance.[52]

### *Mast Cells*

Mast cells are important contributors of type 1 hypersensitivity reactions. Recently, Wingard et al. compared the effects of cerium oxide ($CeO_2$) NP exposure in C57BL/6 mice and mast cell-deficient B6.Cg-Kit(W-sh) mice. Instillation of 100 μg $CeO_2$ NPs (8 nm) activated mast cells, which led to pulmonary inflammation, impaired vascular relaxation, and exacerbated myocardial ischemia/reperfusion injury only in C57BL/6 mice.[53] The authors also observed release of TNF-$\alpha$, IL-6, osteopontin, and prostaglandin $D_2$ from cultured mast cells. Ryan et al. reported that low-dose exposure (10–100 ng/mL) of mast cells to water-soluble C60 fullerenes acted as a negative regulator of release of an IgE-dependent allergic mediator (histamine) leading to suppression of antigen-driven type I hypersensitivity and anaphylaxis.[54] The authors demonstrated the inhibition of oxidative stress and *syk* tyrosine phosphorylation as the possible mechanism involved in this process. This study is particularly important because it proposes or identifies a new role for fullerenes as counteracting agents for mast cell-dependent diseases (asthma, inflammatory arthritis, heart disease, and multiple sclerosis).

NPs can also act as anti-inflammatory agents. Nano-Ag has anti-inflammatory properties, and 1.5 nm Ag NP exposure to peripheral blood mononuclear cells at 10 ppm inhibited the production of cytokines (IL-5, IFN$\gamma$, TNF-$\alpha$) and therefore may have potential in therapy for immunological and inflammatory disorders.[55] $CeO_2$ also shows anti-inflammatory properties and reduced the production/expression of inducible nitric oxide synthase (iNOS) and production of reactive oxygen species from the murine macrophage cell line J774A.1 after low-dose exposure (10 μM).[56] These properties were attributed to a specific structure allowing switching between $3^+$ and $4^+$ oxidation states. Mitchell et al. investigated the effects of multiwalled carbon nanotubes (MWCNTs) on the in vivo immune response and found that MWCNTs could suppress the systemic immune response. The same group reported that signals from the lung-activated signals in the spleen to suppress the immune response and systemic immune dysfunction via the activation of the cyclooxygenase pathway,[57] which was further validated in cyclooxygenase-2 knockout mice.[58] Thus, even without systemic translocation, nanomaterials deposited in distant organs can activate/suppress systemic immune signaling.

## ADAPTIVE IMMUNE RESPONSE

Both innate and adaptive immune responses work in a coordinated manner to mount an effective immune response in the body. As discussed previously, most of the time, the innate immune response is first line of defense against foreign intruders and leads to signals for adaptive immune response activation. The antigens are first taken up and modified by APCs and subsequently presented to lymphocytes (T and B cells), the effector cells. An adaptive response—T and B lymphocyte stimulation and maturation, B-cell class switching to plasmocytes for antibody production—takes

  

several days (2 weeks), but once a response is established, the system can react immediately to a new exposure. With immune suppression, this system will fail to fully expand, thus leading to reduced response on pathogen exposure or failure to recognize mutated cells. In case of immune stimulation, the system will overrespond, thus, leading to allergy and/or autoimmunity. Because of the complexity of an immune response and the multiplicity of possible immune responses, unmixed stimulation or depression is not often seen, but rather an imbalance in the final response is observed. Unfortunately, not many studies have investigated the role of nanomaterials in the adaptive immune system.

An interesting recent study showed that the early innate immune response, to an intra-tracheal single high dose (5 mg/kg) of $TiO_2$ NPs, was followed by long-lasting cell-mediated immune response predominated by $CD4^+$ T cells in the airways of Dark Agouti rats.[32] An early inflammatory response (IL-1$\alpha$, IL-1$\beta$, IL-6, CNIC-1, and GM-CSF) was followed by recruitment of DCs, natural killer (NK) cells and NK T cells at day 2. Of note, the Dark Agouti rat strain is prone to a Th-1-biased immune response involving DCs and macrophage activation. Another important finding was the disruption of alveolar macrophages after engulfment of particles; the authors assumed that clearance of this deposited material toward lymph nodes might have occurred through DCs.

## Route of Administration of NPs and Final Immune Response

Despite many studies on the toxicity of NPs in vivo with different routes of exposure, only a few clear trends can be established.[1] The route of administration dictates the initial phase of the immune response because the final outcome depends to a great extent on the initial players of the immune response. In case of skin exposure, Langerhans cells play a vital role in handling foreign material. In the lung, DCs, alveolar macrophages and the pulmonary epithelium play a crucial role. In the gut, Peyers patches are important immune sensors, whereas in systemic exposure, leukocytes play an important initial role. The importance of these cells might be due to local administration modifying the viability of directly exposed immune cells. Indeed, skin administration of amorphous Si NPs led to loss of viability of epidermal Langerhans cells, which was correlated with NP size (70 nm being more toxic than 300 and 1000 nm) and internalized amount.[59] In another study, only 40-nm polystyrene NPs entered epithelial Langerhans cells and thus were proposed as carriers for the transcutaneous delivery (via hair follicles) of vaccines to cutaneous APCs.[60]

An important consideration is the strength of the interaction of NPs with immune cells, which for macrophages, is stronger with positively charged NPs because of the presence of sialic acid on the macrophage surface. Mumin et al. demonstrated that phosphonate functionalization of mesoporous Si NPs with foam structure (~65 nm) leads to increased levels of interaction or internalization with DCs (bone marrow-derived DCs exposed at 5–5000 ng/mL for 48 h).[61] Rettig et al. described the dependence of size on the nature of the activated immune response by investigating nanometer- or micrometer-sized protamin RNA particles.[62] Nanometer size particles induced IFN-$\gamma$ production, whereas micrometric particles mainly induced TNF-$\alpha$ production in human immune cells. The authors concluded that the immune system distinguishes the size of the associated structure to trigger the adapted antivirus (nanometric) or antibacterial or antifungal (micrometric) immune response.

## NP Effect on Lymphocyte Function and/or Proliferation

Schanen et al. studied the effects of different crystalline forms of $TiO_2$NPs (anatase 7–10 nm, Rutile 15–20 nm) and $TiO_2$ nanotubes (10–15 nm wide and 70–150 nm long) in vitro using a human immune construct (Mimic). NP treatment (1.56 μM for 24 h) led to pro-inflammatory (innate) cytokine production [IL-6, IL-8, TNF-$\alpha$, IL-1a, IL-1b, interferon $\gamma$ (INF-$\gamma$)], DC maturation ($CD86^+$, $CD83^+$, and $CCR7^+$ expressions) and activation and proliferation of naïve $CD4^+$ T cells.[63] As well, Ghoneum et al. showed in vitro that 24-h exposure to dispersions containing 50–200 μg/mL nanodiamonds or nanoplatinum (DPV576) activated human monocyte-derived DCs ($CD86^+$ and $CD83^+$ expressions), which led to production of IL-6, TNF and IL-10 and subsequently stimulated or activated (increased $CD25^+$ expression) the proliferation of $CD4^+$ naïve T cells.[64] The authors proposed that these nanomaterials may be used to boost the immune response in cancer treatment. Ogunwale et al. found some dissimilar effects of 4 nm Co-chromium (CoCr) NPs on DCs and T and B lymphocytes.[65] DCs and B lymphocytes ($CD40^+$ and $CD86^+$ expressions, respectively) were not affected by these NPs (25 μg/mL), and the proliferation of T lymphocytes [in response to signal 1 ($CD3^+$) and signal 2 ($CD3^+$ and $CD28^+$)] was reduced. However, whether the dissolution of the material played a role in these observations was unknown.

  

As compared with naked DNA, DNA adsorbed to cationic polystyrene NPs (poly-L-lysine-coated) can, after intradermal administration, enhance antibody production and $CD4^+$ and $CD8^+$ T cell proliferation.[66] The authors further observed that the optimal size of poly-L-lysine-coated polystyrene NPs for optimal delivery of DNA vaccine was 0.05 μm. The absorbed material, as well as the size of the particles, plays a role because potent $CD8^+$ T cell activation and Th-1 response was observed with 40–50 nm polystyrene NPs, whereas larger particles (>500 nm) preferentially induced a Th-2 response.[67,68] Zogovic et al. demonstrated that C60 fullerenes (0.0625–1 μg/mL for 48 h) in vitro caused apoptotic and necrotic cell death in mouse B16 melanoma cells because of the induction of oxidative stress and mitochondrial depolarization. However, in vivo, intraperitoneal administration of 5 μg C60 significantly augmented tumor growth.[69] Besides the tumor-promoting effect of C60, splenocyte production of NO was significantly increased and proliferative capacity of T and B cells was reduced. The latter effect could be important in the tumor-promoting effects observed.

## NPs and Allergy

Various types of ultrafine particles (UFPs), such as CB, diesel exhaust particles (DEP) matter, and atmospheric UFP, have allergy adjuvant effects,[20,61,68] but such reports of engineered NPs are few. CB NPs (50 μg/mouse) have a size-dependent effect (14 nm more potent than 56 nm) and induce the lung expression of GM-CSF, macrophage inflammatory protein 1 $\alpha$, IL-2, and IL-10 as compared with allergen exposure alone.[70] In contrast, fullerenes have anti-allergic effects and significantly inhibit IgE-dependent mediator release.[54]

Bezemer et al. studied the effects of ambient and engineered NPs on the adaptive immune response in mice.[71] The authors compared the effects of ambient PM (aPM) exposure to that of DEP, CB particles and Ag NPs. After pulmonary dosing, aPM and DEP activated DCs and provoked a T-helper cell type 2 (Th2) response in a mouse model. Naïve to prior PM exposure, purified lung DCs were studied for their activation after oropharyngeal instillation of particles ex vivo. The engineered CB and Ag NPs altered the lung tissue barrier integrity but failed to stimulate $CD4^+$ T cells.[71] Kioke et al. studied the effects of CB NPs on mouse bone marrow-derived DCs to elaborate the possible mechanism of adjuvant effects on allergic disorders.[72] CB NPs significantly affected functioning and maturation or differentiation of murine bone marrow-derived DCs (increase in expression of $CD205^+$ and $CD86^+$ cells and increase of MHC class II and $CD80^+$ expression, although not significantly). Of note, the same authors failed to observe a size-dependent effect for these parameters but did observe a size-dependent (14 versus 56 nm) enhancement of mixed leukocyte reaction (MLR). The same group previously demonstrated that pulmonary exposure to 14 nm CB NPs could increase the expression of MHC II and costimulatory molecules. Moreover, the number of APCs in the lung increased, especially in the presence of antigen, which resulted in subsequent antigen-related airway inflammation and immunoglobulin production.[73]

Larsen et al. demonstrated in a mouse model of asthma [sensitization to the protein ovalbumine (OVA)] that intraperitoneal sensitization with OVA and $TiO_2$ (28 nm, 250 μg), followed by OVA aerosol challenge induced a significantly higher level of OVA-specific IgE than the standard OVA/alum adjuvant sensitization followed by OVA challenge.[74] Furthermore, OVA and $TiO_2$ sensitization led to the same Th2 response (bronchoalveolar eosinophils, lymphocytes, IL-4, IL-5, and IL-10), as compared with OVA and alum sensitization. Therefore, $TiO_2$ could be considered an adjuvant in these experiments. Nygaard et al. demonstrated the allergy-promoting effects of CNTs in mice and showed differences between SWCNTs (4-nm wide and 0.5–100-μm long) and MWCNTs (15-nm wide and 0.5–200-μm long).[75] Both SWCNTs and MWCNTs (total dose 200-μg injection model and 400 μg in 3-day exposure), together with OVA, strongly increased serum levels of OVA-specific IgE, number of eosinophils in bronchoalveolar fluid, and secretion of Th2-associated cytokines. However, only MWCNT and ultrafine CB exposure in the OVA-model increased the number of neutrophils, as well as IgG2a level, TNF-$\alpha$ and MCP-1 levels in bronchoalveolar fluid, which corresponded with Th1 promotion.

## NPs and Immune-Sensitive Lung Disorders

Because NPs are produced in large quantities for industrial and consumer use, the possibilities of modulating immune-sensitive disorders cannot be ruled out, especially for occupationally exposed populations. Workers exposed to different types of nanomaterials are also susceptible to coexposure to other occupational contaminants. Moreover, as discussed below, some occupational agents such as diisocyanates can lead to chemical-induced occupational asthma, with the sensitization and disease symptoms persisting years after removal from the causative agent.[76–78]

  

Therefore, exposure of sensitized individuals to NPs is potentially hazardous and needs to be explored (Box 3).

**BOX 3**

**ANIMAL MODELS FOR ASTHMA**

Although no mouse model is currently able to mimic the full range of clinical manifestations of human asthma, a number of models reproduce important features that characterize its most common phenotypes, including airway hyperreactivity (AHR) and pulmonary inflammation.

Most animal models, are developed to study allergic asthma, and use biological agents with a relative high-molecular-weight (HMW), the most common used molecule is OVA but other proteins or pollen have also been used (refer to recent reviews by Ref 79); far fewer models have been developed for chemical-induced [low-molecular-weight (LMW) agents] asthma (refer to recent reviews by Ref 80).

As compared with asthma induced by HMW agents, where eosinophils and lymphocytes are the characteristic cell types present in the bronchoalveolar lavage fluid, asthma induced by LMW agents has been associated with an influx of mainly neutrophils and eosinophils.

Independent of the compound used to induce the asthmatic phenotype, all animals undergo a sensitization prior to the challenge inducing hyperreactivity. During sensitization, the animals are exposed to the compound/antigen, often via different routes, to allow the adaptive immune system to generate a specific response to the antigen. In articles describing an asthmatic response on exposure to NPs, these NPs were never applied as an antigen but were applied as supplementary stimulus.

Several studies have described the effects of NPs on LPS-induced airway dysfunction (mimicking disorders of the innate immune system). Various types of NPs (CB, $TiO_2$, latex, and CB nanotubes) aggravate the LPS-induced airway abnormality (edema, inflammation, and increased production of cytokines and chemokines).[70,72,73,81–85] Also, ambient DEP had an aggravating effect on allergic asthma in an OVA mouse model.[86,87] However, the effect of NPs on modulating lung disease has rarely been investigated. We recently assessed the effects of $TiO_2$ and Au NPs on the modulation of the asthmatic response in a mouse model of diisocyanate-induced asthma,[88] which was previously developed and validated in our laboratory.[89–92] Isocyanates are the leading cause of chemical-induced occupational asthma worldwide.[93] They are used in the production of polyurethane, which has diverse industrial applications (production of foams, durable elastomeric wheels and tires, automotive suspension bushings, electrical potting compounds, high performance adhesives and sealants, seals, gaskets, carpets). Occupational exposure to toluene diisocyante (TDI) can occur during production, handling and use,[94] and emissions from urethane production facilities can lead to exposure in the general population.[95] TDI-induced asthma shares similarities with common environmental asthma (natural history, clinical symptoms, and pathology) but also differences (minimal Th2 response, minimal association of atopy, allergen-specific IgE and serum levels of total IgE).[96] In this model, mice were dermally sensitized to TDI on days 1 and 8 and oropharyngeally challenged with NPs on day 14. Both NP-exposed and NP-unexposed mice were challenged with the asthmogen (TDI) on day 15, and endpoints were assessed 1 day later. The results confirmed the abilities of both types of NPs ($TiO_2$ and Au) to increase airway hyperreactivity (AHR) and lung inflammation. The aggravating effects were accompanied by increased secretion of MCP-1 and MMP-9. The percentage of macrophages engulfing NPs was significantly increased in sensitized and challenged animals. These results are the first to confirm the stimulatory effect of asthma in a mouse model by the concomitant exposure of NPs.

## MECHANISMS OF IMMUNE MODULATION

A delicate balance between the innate and adaptive immunity exists and is important, for example, in cancer immunity and avoiding hypersensitivity reactions. NPs can interact with several innate and acquired immune cells/functions, but the mechanisms, however, remain unclear (Figure 2).

NP-induced oxidative damage could be one of the leading factors of the immune imbalance because oxidative stress plays an important role in the pathogenesis of allergy and asthma, and many types of NPs have been shown to produce oxidative stress under both in vitro and in vivo conditions.[38,88,97–99] Other possible mechanisms might be particle-induced epithelial damage to the respiratory barrier, which leads to increased susceptibility to allergens.[100,101] In line with this, MMP-9 was found in vitro to modulate the tight junction integrity of the airway epithelium, thereby initiating lung tissue remodeling.[102] We showed increased levels of MMP-9 in a murine in

  

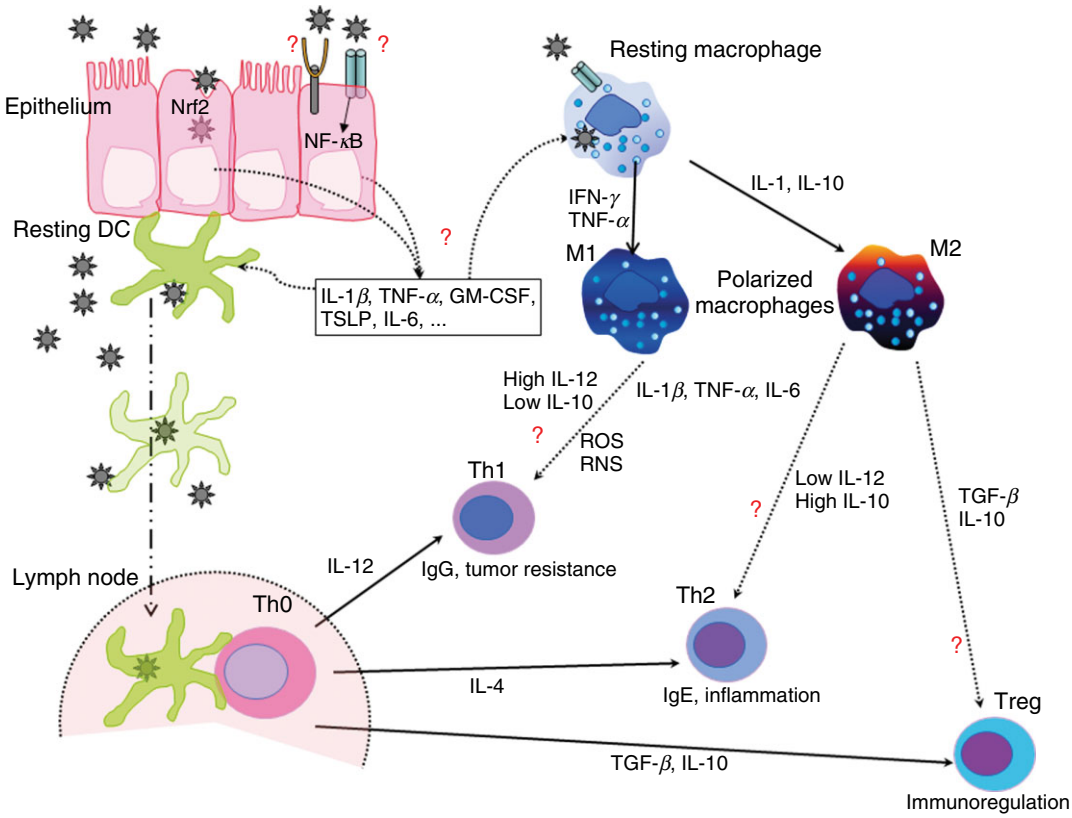


**FIGURE 2** | Exposure to nanoparticles (NPs, ✱) will effect different cells at the side of exposure. Epithelial cells, tissue macrophages, and dendritic cells will respond simultaneously to an exposure. The final response will depend on the nature of the particle and the 'mixed' signals from the cells involved. After the initial stimulation—direct via particle exposure or indirect via released mediators—dendritic cells and macrophages will develop into mature cells and affect both the innate and adapted immune response. In case of preexisting sensitization, NP exposure can affect the exacerbation during allergen challenge. In case of an allergen challenge without preexisting sensitization the developing allergy can be affected with coexposure to NPs. Th1, type 1 T-helper lymphocyte; Th2, type 2 T-helper lymphocyte; Treg, regulatory T-lymphocyte; M1 & M2, polarized macrophages type 1 & 2; DC, dendritic cell; , toll-like receptor (TLR); , receptor of the complement sytem; ROS, reactive oxygen species; RNS, reactive nitrogen species; Nrf2, Nuclear factor (erythroid-derived 2)-like 2; NF-κB, nuclear factor kappa B. Arrows: dotted line, possible influence; broken line, migration; full line, polarization/differentiation.

vivo model of asthma with exposure to $TiO_2$ or Au NPs as compared with nonexposed asthmatic mice.[88]

Recent reports describe that nanomaterials can affect the Th1/Th2 balance (adaptive immune response). Coexposure of NPs ($10^{-5}$–$10^{-7}$ M) differentially influenced the cytokine production in peripheral blood mononuclear cells and led to overproduction of TNF-α and INFγ, whereas levels of IL-10 and IL-2 were decreased.[103] Water-soluble C60 fullerenes (0.5 μmol/kg) induced the Th1 immune response (increased IL-2, INF-γ and TNF-α production) and decreased the Th2 response (decreased IL-4, IL-5, and IL-6 production) in serum of mice. The authors concluded that fullerenes have efficient antitumor activity associated with increased CD+4/CD+8 lymphocyte ratio and the enhancement of TNF-α production.

An important mechanism that might be responsible for nanomaterial-induced immunotoxicity is their ability to induce or modify the maturation and differentiation of DCs. This property has been used to engineer novel vaccines containing nanomaterials to increase adjuvant properties. Colloidal Au (17 nm) NPs have shown promise.[104] Furthermore, Park et al. demonstrated that a single intra-tracheal administration of high-dose (1 mg/kg) platinum NPs (20–30 nm) increased the concentration of pro-inflammatory cytokines (IL-1, TNF-α, and IL-6), Th0 cytokine (IL-2), Th1-type cytokine (IL-12), and Th2-type cytokines (IL-4 and IL-5); the induction of Th2-type cytokines was higher than that of Th1-type cytokine on day 28 after instillation.[105]

## CONCLUSIONS AND PERSPECTIVES

A delicate balance between innate and adaptive immunity is required for efficient functioning of

  

the immune system. This balance is important in cancer immunity, immune response against pathogens, and avoiding hypersensitivity reactions. NPs can imbalance both the innate and the adaptive immune system, but mechanistically functions on how NPs modulate the immune system and the diseases resulting from this imbalance remain unclear (Figure 2).

Considering the innate immunity, some nanomaterials induce a (pro)-inflammatory response and are taken up by phagocytic cells, whereas others seem to reduce these activities reducing the ability of these immune cells to fight (e.g., bacteria). Unfortunately, predicting the innate response (in vitro or in vivo) of NPs is still difficult.

Considering the adaptive immune system, additional endpoints should be considered in the strategies for NP evaluation, with particular emphasis on the disruption of the T cell balance (Th1, Th2, Th17, Treg), which could be a first danger signal.

Keeping in view the huge significance of nanomaterials, surface coating could be a valuable strategy to avoid possible overt immune response after NP exposures. Polyethylene glycol (PEG), is commonly used to avoid NP recognition as 'foreign' by immune system. However, potential problem associated with this strategy includes the formation of PEG-specific antibodies leading to altered kinetics of NPs after successive exposures as it was shown for liposomes.[106–108]

More systematic studies are needed, particularly those controlling the characteristics of the nanomaterials and exposing cells and/or full organisms at low (realistic) concentrations, which would result in only mild responses (mild inflammation, low toxicity), to study chronic exposure situations. To avoid huge numbers of in vivo studies, more effort should be undertaken to use multiple cells in one culture system, thus allowing 'natural' interactions between cells.[109] Finally, establishing pertinent endpoints and testing guidelines for immunological evaluation of nanomaterials are desired.

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