Metal nanomaterials: Immune effects and implications of physicochemical properties on sensitization, elicitation, and exacerbation of allergic disease

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ABSTRACT

The recent surge in incorporation of metallic and metal oxide nanomaterials into consumer products and their corresponding use in occupational settings have raised concerns over the potential for metals to induce size-specific adverse toxicological effects. Although nano-metals have been shown to induce greater lung injury and inflammation than their larger metal counterparts, their size-related effects on the immune system and allergic disease remain largely unknown. This knowledge gap is particularly concerning since metals are historically recognized as common inducers of allergic contact dermatitis, occupational asthma, and allergic adjuvancy. The investigation into the potential for adverse immune effects following exposure to metal nanomaterials is becoming an area of scientific interest since these characteristically lightweight materials are easily aerosolized and inhaled, and their small size may allow for penetration of the skin, which may promote unique size-specific immune effects with implications for allergic disease. Additionally, alterations in physicochemical properties of metals in the nano-scale greatly influence their interactions with components of biological systems, potentially leading to implications for inducing or exacerbating allergic disease. Although some research has been directed toward addressing these concerns, many aspects of metal nanomaterial-induced immune effects remain unclear. Overall, more scientific knowledge exists in regards to the potential for metal nanomaterials to exacerbate allergic disease than to their potential to induce allergic disease. Furthermore, effects of metal nanomaterial exposure on respiratory allergy have been more thoroughly-characterized than their potential influence on dermal allergy. Current knowledge regarding metal nanomaterials and their potential to induce/exacerbate dermal and respiratory allergy are summarized in this review. In addition, an examination of several remaining knowledge gaps and considerations for future studies is provided.

Introduction

Over the past several decades, an extensive amount of scientific attention has been invested in the field of nanotechnology. Significant advances have been made in understanding the unique behaviors of matter with nano-scale dimensions. This progress has facilitated the capacity for manipulation of material properties to optimize their functional utility. Subsequently, nanomaterials have proven useful in diverse applications ranging from pharmaceutics and energetics to transportation and electronics. The exponential growth of the nanotechnology field has left few sectors unaffected by its momentum, as the global nanotechnology market has been valued at over $20 billion US (Nanomaterials Future Markets 2015). Although the resounding impact of these technological advancements has generated comparisons to the impact of the industrial revolution, the expansion of nanotechnology has also generated several notable concerns. In addition to the environmental, legal, ethical, and regulatory challenges imposed by the expanding presence of nanotechnology, the potential risk for adverse health effects following exposure to nanomaterials has also become a major concern.

As a result, a unique discipline of toxicology has emerged to evaluate the potential health effects of nanomaterials. Nanotoxicology studies have consistently demonstrated that the unique properties of nanomaterials that render their industrial functionality also implicate unique interactions with biological systems. A general correlation between decreasing size and increased toxic potential has been observed for many nanomaterials (Shang et al. 2014). However, additional physical and chemical properties of nanomaterials have been implicated in their biological activity. Nanomaterials exist in various morphologies (Figure 1) with diverse surface textures, and can assume differing degrees of agglomeration. These physical properties contribute to variations in their chemical properties, which include surface charge, dissolution kinetics, and surface reactivity (Oberdörster et al. 2005; Castranova 2011; Gatoo et al. 2014).

One of the greatest challenges presented to nano-toxicologists arises from the discord between the rapid emergence of vast quantities of new nanomaterials and the significant amount of time and resources required to evaluate the safety of each material individually. A novel risk assessment approach proposed to...
mitigate this issue involves delineation of relationships between specific physicochemical properties and toxicological modes of action (Kuempel et al. 2012; Braakhuis et al. 2016). Subsequently, emerging materials can be categorized by this scheme, providing preliminary safety information and prioritization of resources for in vivo studies (Schulte et al. 2014). Significant advancements have been made using this approach with respect to toxic effects on the lungs, but the correlation of nanomaterial physicochemical properties with adverse effects on other systems, such as the immune system, are less clear.

In addition to protecting the host from both endogenous and exogenous threats, the immune system is a critical regulator in hundreds of other disorders, as inflammation is a critical component in the pathophysiology of nearly all chronic diseases states (Pawelec et al. 2014). Accordingly, deviations in optimal immune functioning can have resounding effects on host health, whether polarized towards being either stimulatory or suppressive in nature. One of the immunological disorders presenting a significant and continually expanding global public health burden is allergy. The term “allergic disease” refers to a collective assortment of disorders involving diverse inciting agents, underlying immunological mechanisms, and clinical manifestations. However, all hypersensitivity disorders are characterized by commonality in hyperactivation of adaptive immune responses directed at otherwise innocuous exogenous antigens (Pawankar 2014).

Rates of allergic disease have been on the rise for decades, and the American Academy of Allergy, Asthma, and Immunology reports that worldwide, sensitization rates to one or more common allergens are approaching 40–50% in school-aged children (AAAAI 2015). In the United States, allergic diseases are the sixth leading cause of chronic illness with an annual cost exceeding $18 billion US (Centers for Disease Control and Prevention 2017). Although the development of allergy is dependent on a multitude of genetic, behavioral, and environmental factors, exposures to immunotoxic agents are a major

Figure 1. Different morphologies of nanomaterials are shown: (a) graphene sheets, (b) silver nanoparticles, (c) silver nanowires, (d) gold nanorods, (e) gold nanoparticles, (f) nickel oxide nanoparticles, and (g) copper oxide nanoparticles.
Mental nanomaterials, various applications, and reported global production volume for 2014, reported in tons. Adapted from Metal and Metal Oxide Nanomaterials Future Markets Report, Table 1 (Nanomaterials Future Markets 2015).
**Metals and dermal allergy**

The most common metal-induced allergic disorder of the skin is ACD, a T-cell-mediated delayed-type hypersensitivity response. Dermal sensitization and the subsequent induction of ACD requires several key molecular and cellular events (Figure 2), which have been outlined in an adverse outcome pathway (AOP) by the Organization for Economic Co-operation and Development (OECD) (OECD 2014).

The preliminary requirement for skin sensitization is bioavailability of the sensitizing agent. Since a primary function of the skin is to serve as an effective barrier between the host and environment, the sensitizing potential of many antigens is limited by their capacity to evade this barrier (Jaitley and Saraswathi 2012). Passage through the uppermost layers of the epidermis is heavily dependent on antigen physical and chemical properties. Likewise, most dermal sensitizers tend to be low molecular weight (LMW, <500 Daltons) chemicals with adequate lipophilicity (logP <2) (Chilcott and Price 2008; Karlberg et al. 2008). Metals associated with skin sensitization present the greatest concern when formulated as soluble salts that release ions capable of penetrating the physical barrier presented by the epidermis (Di Gioacchino et al. 2007; Kubo et al. 2013).

The next steps in the skin sensitization AOP involve the molecular initiating event of skin sensitization-antigen formation. The small size required for antigen passage through the stratum corneum is not conducive with cellular recognition (Anderson et al. 2011). As a result, most skin sensitizers are referred to as haptens, which must acquire or possess inherent chemical reactivity that facilitates binding to carrier molecules (Büding et al. 2000; Chipinda et al. 2011). This process generates adequate size for recognition by an antigen-presenting cell (APC). The APC most frequently implicated in dermal sensitization is the resident dendritic cell (DC) of the epidermis, the Langerhans cell (LC) (Thyssen and Menne 2010).

In addition to uptake of the hapten/carrier complex, activation of LC requires an additional antigen-nonspecific signal indicative of an elevated threat level. Many mediators capable of fulfilling this signal are released by non-immune cells including keratinocytes in response to injury (Dearman and Kimber 2003). Presence of both antigen-specific and nonspecific signals induce LC maturation, upregulation of co-stimulatory molecules, antigen processing, and migration to the lymph nodes (Tončić et al. 2011). Once the LC reaches the lymph nodes, the processed hapten is presented via major histocompatibility Class I (MHC I) molecules to naïve CD8+ T-lymphocytes until recognition occurs by antigen-specific T-cell receptor (TCR). Given adequate costimulatory signals from the LC, T-lymphocytes undergo proliferation producing a pool of clonal antigen-specific effector T-cells. The T-cells enter the circulation, and following resolution of inflammation, a subset of these effector cells will survive and become memory T-cells, completing the process of sensitization.

Upon future exposures to the allergen, memory and effector T-cells are recruited to the site of exposure where CD8+ T-cells exhibit immediate cytotoxic effector functions. CD4+ T-helper (T_{H}1) of the T_{H}1 phenotype have regulatory roles in ACD and produce high levels of the cytokines interleukin (IL)-2 and interferon (IFN)-γ, contributing to inflammatory cell recruitment (Sasseville 2008; Tončić et al. 2011). Within 48 h, the inflammatory process originally orchestrated to destroy the antigen results in the clinical manifestations of ACD, including localized skin redness, swelling, and itching at the site of allergen contact.

Metals are among the most common inducers of ACD in the general population. Patch test studies have generated data from thousands of subjects and reveal that the most common inducers of metal ACD are nickel, gold, cobalt, and chromium (Belloni Fortina et al. 2015). Interestingly, studies using subjects exclusive various geographical locations have demonstrated that these four metals are consistently problematic with respect to ACD worldwide (Kanerva et al. 2000; Mattila et al. 2001; Goon and Goh 2005; Cheng et al. 2008; Davis et al. 2011; Nonaka et al. 2011; Khatami et al. 2013; Mahler et al. 2014; Kim et al. 2015; Malinauskienė et al. 2015; Linauskiene et al. 2017). Though less frequently associated with ACD, copper, aluminum, and platinum group metals are also known to cause skin allergy in some individuals (Hostynek et al. 1993; Bergfors et al. 2005; Faurshou et al. 2011; Fage et al. 2014).

**Metals and respiratory allergy**

Metals are also associated with respiratory allergy and IgE-mediated asthma. An AOP specific to the events of respiratory sensitization has not been adopted by the OECD, but many of the same steps of the dermal sensitization AOP are involved in the development of asthma. Accordingly, bioavailability of the
sensitizing agent is also a preliminary limiting factor in respiratory sensitization potential. Since the primary function of the respiratory tract is to facilitate gas exchange between the host and environment, it is particularly vulnerable to adverse effects from a diverse assortment of agents in the inhalable (<20 μm) and respirable size range (<10 μm) (Elder and Oberdörster 2006). Likewise, bioavailable metals capable of sensitizing the respiratory tract are not limited to ions, like in the skin (Linde et al. 2017). Respirable metals may be encountered as particulate matter, vapors, or fumes and can be constituents of compounds including oxides, sulfides, and salts, or as complexes with ammonia, carbon monoxide, and organic nitrogen (Malo et al. 2013).

Since both low and high molecular weight (HMW) agents are capable of inducing asthma, the molecular initiating events of respiratory sensitization may differ accordingly. Similar to the skin, pulmonary immune responses following inhalation of metals can be nonspecific and self-limiting, or can result in the recruitment of the adaptive immune system. Lung-resident DC take up antigen, and given adequate second signals, the antigen is processed and DC migrate to the lung-draining lymph nodes. Here, the peptide is presented to naive CD4+ T-lymphocytes along with costimulatory molecules, resulting in the preferential expansion of the TH-2 phenotype lymphocytes. These cells produce high levels of IL-4, IL-5, and IL-13, and stimulate isotype switching and allergen-specific IgE-production by B-cells. The Fc portion of secreted IgE is bound to FcεRI receptors present on tissue-resident mast cell surfaces and circulating basophils, exposing the antigen-recognizing motif, completing the sensitization process (Verstraeten et al. 2008).

Upon subsequent encounters, the allergen is bound by allergen-specific IgE on the surface of mast cells and basophils. Binding induces crosslinking of receptors and the subsequent release of preformed mediators such as histamine, beginning the anaphylactic cascade responsible for the early asthmatic reaction experienced minutes after antigen encounter. Acute clinical manifestations of allergic asthma range from rhinitis and bronchoconstriction to anaphylactic shock. The late phase asthmatic response occurs 4–6 h later as a result of mast cell mediators and recruitment of inflammatory cells (Possa et al. 2013). Clinical presentations of the late phase asthmatic response tend to be more severe than early phase responses, and include excessive mucus production, increased vascular permeability, and airway constriction. Chronic cycles of allergic inflammation and subsequent repair are associated with structural alterations in the airways that can have physiological implications, such as a decline in lung function (Erle and Sheppard 2014).

Compared to metal-induced ACD, metal-induced asthma occurs far less frequently. Cases tend to be isolated to individuals working in occupations involving metalwork where metal fumes, dust, or vapors are generated and inhaled (Wymann and Hines 2018). Nickel, chromium, cobalt, vanadium, zinc, platinum and aluminum have all been associated with cases of occupational asthma (Musk and Tees 1982; Hong et al. 1986; Malo et al. 2013). However, metal-specific IgE has only been implicated in cases caused by nickel, platinum, chromium, and cobalt (Malo et al. 1982; Murdoch et al. 1986; Shirakawa et al. 1988, 1990, 1992; Kusaka et al. 1996). Metal-specific IgG molecules have also been implicated in cases of cobalt and platinum-induced asthma (Pepys et al. 1979; Ciria 1994). The presence of metal-specific IgE has also been confirmed in the absence of asthmatic symptoms, emphasizing the potential for numerous immunological mechanisms in metal-specific asthma (El-Zein et al. 2005; Turcić et al. 2013).

**Metals and allergy adjuvancy**

In addition to their potential to induce sensitization, metals are also associated with the capacity to modulate allergic responses to nonmetal allergens. Contrasted with the consistent, sequential series of cellular processes involved in allergic sensitization and elicitation, adjuvant effects can emerge as a result of various mechanisms in various phases of allergic disorders (Figure 3).

An example of metal adjuvant effects on the development of adaptive immune responses is best demonstrated by aluminum hydroxide, which is one of the most frequently used vaccine adjuvants. When administered with poorly-immunogenic antigens, aluminum hydroxide induces adequate stimulation of the innate immune system to generate antigen-specific immunological memory. Mechanisms of immunopotentiation associated with aluminum hydroxide include triggering release of alarmins, activation of inflammasomes (intracellular multi-protein complexes involved in innate immune responses), and DC activation; however, numerous other mechanisms including immune cell recruitment and activation, modulated cytokine production, and altered antigen delivery kinetics, can also enhance sensitization (Naim et al. 1997; Aimanianda et al. 2009). Similarly, metals are also associated with adjuvant effects on established allergic conditions, as demonstrated by metal-rich

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**Figure 3.** Potential adverse outcomes with respect to the sensitization and elicitation phases of allergy following exposure to immunotoxic agents. Adjuvant effects resulting from exposure prior to allergen sensitization can manifest as increased susceptibility to sensitization. Exposure concurrent to sensitization may lower the threshold of allergen exposure required to induce sensitization. Following sensitization to allergen, exposure to an immunotoxic agent either in the absence or presence of allergen may result in a lower threshold of exposure required to induce elicitation reactions or increased severity of elicitation symptoms. These effects may further increase susceptibility to elicitation reactions as result of physiological alterations such as compromised skin barrier integrity. Furthermore, isolated exposure to immunotoxic agents or concurrent to allergens in established allergic disease conditions may also contribute to the progression of chronic effects, such as airway remodeling, which can also further contribute to elicitation reactions.
ambient air pollution, which is known to exacerbate the severity of asthmatic responses to environmental allergens (Gavett et al. 2003; Schaumann et al. 2004). Adjuvant effects on allergic elicitation can involve mechanisms including induction of pulmonary oxidative stress, enhanced degranulation of mast cells, and recruitment of inflammatory cells (Walczak-Drzewiecka et al. 2003; Ghio 2008).

**Unique properties associated with metal immune effects**

Metals are known to induce many unique immune effects implicated in allergic disease. With respect to sensitization, the modulation of innate immune reactivity by some metals has been associated with their immuno-genicity. For example, some metal ions are known to produce functional mimicry of pathogen-associated molecular patterns (PAMP) (Schmidt and Goebeler 2015). Gold ions have the capacity to bind and activate Toll-like receptor (TLR)-3, while nickel, cobalt, and palladium ions have the capacity to bind and activate human TLR-4 (Schmidt et al. 2010; Rachmawati et al. 2013, 2015). The subsequent induction of pro-inflammatory signaling generates the antigen nonspecific signals required for DC activation, promoting sensitization.

Metals are also known to modulate mechanisms of communication between innate and adaptive immune cells. Accordingly, antigen presentation is another step in allergic sensitization that is subject to interference by metals. Beryllium and noble metals have been shown to induce structural alterations in MHC molecules, impacting subsequent interactions with TCR (de Wall et al. 2006; Falta et al. 2013). Similarly, peptide-independent linking of MHC and TCR by nickel has been demonstrated (Gamerdinger et al. 2003; Thiére et al. 2005). Adaptive immune responses can also be affected by metals, as demonstrated by CD4+ nickel-specific T-cell clones, which were shown to cross-react when presented with other transition metals including copper and palladium (Moulon et al. 1995; Pistoor et al. 1995).

Many of the unique immune effects associated with metals emerge as a result of their capacity to alter molecular and cellular interactions on a biochemical level. Accordingly, their modulation of processes involved in allergic disease is critically dependent on physicochemical properties including special geometry, oxidation state, and solubility (Schuhmann et al. 1990; Kinbara et al. 2011). Many of these properties are altered in nanoparticulate form, suggesting that metal nanomaterials may exhibit novel mechanisms of immune interaction with implications for allergic disease.

**Metal nanomaterials and dermal hypersensitivity**

The potential for adverse immune effects following dermal exposure to metal nano-materials is a growing concern due to their increasingly frequent incorporation into consumer goods intended to have prolonged contact with the skin (Yang and Westerhoff 2014). The unique optical properties of titanium dioxide nanoparticles (TiO2 NP) and zinc oxide nanoparticles (ZnO NP) have led to their incorporation in sunscreens and cosmetics for their protective effects against ultraviolet radiation (UVR) (Smijis and Pavel 2011; Yoshioaka et al. 2017). Silver nanoparticles (AgNP) are being incorporated into clothes, medical textiles, toys, and cleaning products due to their antimicrobial properties, and silica-based nanoparticles (SiNP) have been frequently used in cosmetics and as a coating material to alter the properties of other materials (Contado 2015; Tulve et al. 2015). Likewise, the dermal effects of TiO2 NP, ZnO NP, AgNP, and SiNP are a particular concern with respect to the general public (Weir et al. 2012). These nanomaterials are also a concern for workers, but other metal nanomaterials with high rates of production (listed in Table 1) are also associated with dermal exposures in the workplace.

The potential for metal nanomaterials to penetrate the skin, induce dermal sensitization, and modulate skin allergy development/responses are the three main areas discussed in this section with respect to size and other physicochemical properties. In correspondence with the review of the literature, Table 2 summarizes studies characterizing effects of individual metal nanomaterials on skin allergy. Table 3 summarizes studies designed to examine effects of physicochemical properties of metal nanomaterials on dermal allergy. Table 4 highlights key events involved in dermal sensitization and elicitation that have been shown to be subject to modulation by metal nanomaterials and their corresponding physicochemical properties.

**Skin penetration and translocation studies**

Adverse immune effects following dermal exposure to an agent are heavily dependent on the degree to which the skin protects from its entry into the body. Likewise, one mechanism by which dermal exposure to metal nanomaterials may lead to increased potential for adverse immune effects compared to larger-sized metals is by size-mediated evasion of skin barrier function. Although it seems logical that the small size of nanomaterials would inherently provide increased opportunity for absorption via the skin, there is currently no general consensus on the skin-penetrating capabilities of nanomaterials as a collective class of agents (Vogt et al. 2006; Baroli 2010; Try et al. 2016).

Numerous studies have demonstrated that metal nanomaterials (<100 nm) can penetrate skin in various in vivo and in vitro models. Iron-based nanoparticles (FeNP), gold nanoparticles (AuNP), palladium nanoparticles (PdNP), nickel nanoparticles (NiNP), AgNP, SiNP, and metal-based quantum dots (QD) have all been associated with penetration of the skin (Baroli et al. 2007; Chu et al. 2007; Filon et al. 2011, 2016; Labouta et al. 2011; Hirai et al. 2012a; Rancan et al. 2012; George et al. 2014; Crosena et al. 2016; Kraelsing et al. 2018). Moreover, many of these studies have established a relationship between decreased particle size and increased potential for skin permeation (Rymann-Nasmussen et al. 2006; Sonavane et al. 2008; Matsuo et al. 2016; Raju et al. 2018). Hydrophobicity, surface charge, and morphology are additional properties that have been shown to be influential in the capacity for these nanomaterials to pass through the stratum corneum (Rancan et al. 2012; Lee et al. 2013; Iannuccelli et al. 2014; Fernandes et al. 2015; Tak et al. 2015; Mahmoud et al. 2018).

By comparison, the majority of studies investigating the skin-penetrating potential of metal nanomaterials have been conducted with ZnO NP and TiO2 NP and have not generated equally consistent findings. Numerous studies have demonstrated that the stratum corneum effectively restricts passage of TiO2 NP, irrespective of size shown to facilitate penetration of the skin by other metal nanomaterials. Repeated application of different forms of TiO2 NP did not lead to skin penetration in hairless rats, elevated levels of titanium in lymph nodes of minipigs, or penetration of human skin transplanted onto immunodeficient mice (Kiss et al. 2008; Sadrieh et al. 2010; Adachi et al. 2013). Although TiO2 NP were shown to accumulate in and around furrows of the skin, microscopic analysis was used to confirm that 20–100 nm TiO2 NP remained restricted to the
| Metal     | Author/year | Material | Size     | Animal or cell type | Model     | Exposure route | Dose                | Findings                                                        |
|-----------|-------------|----------|----------|--------------------|-----------|----------------|---------------------|----------------------------------------------------------------|
| Aluminum  | Varlamova et al. 2015 | Al2O3   | –        | M/F BALB/c, CBA/CaLac, outbred mice and guinea pig | LLNA      | Subcutaneous, intramuscular, intravenous, intradermal injection | –                   | No intensification of anaphylaxis systemic reaction, no inflammatory reaction to ConA, no delayed allergic reaction, no redness or edema at site of application |
|           | Brown et al. 2008 | Al2O3    | 50–120 nm | HaCaT human keratinocytes | In vitro | –              | 10–10,000 µg/mL     | 24 h exposure resulted in IL-8 expression, IL-1α release, indicating potential for irritation or sensitization |
| Cobalt    | Cho et al. 2012 | Co3O4   | 18.4 ± 5.0 nm | F C57BL/6 mouse | OVA | Subcutaneous injection | 25 µg              | Balanced Th1/Th2 response when used as adjuvant, causing higher specific IgG2c and IgG1 and less IgE |
| Gold      | Ishii et al. 2008 | Au      | 5.2 ± 1.3 nm | F Japan White Rabbit | –        | Intradermal injection | 1 mg               | Azobenzene dye hapten conjugation to AuNP led to high yield of IgG specific to the agent indicating the capacity for AuNP to act as both a carrier and adjuvant |
| Iron      | Shen et al. 2012 | Fe3O4   | 58.7 nm   | M BALB/c mouse | OVA      | Intravenously     | 0.2–10 mg/kg        | Decreased footpad swelling, infiltration of macrophages and T-cells, and IFN-γ, IL-6, TNF-α levels |
|           | Hsiao et al. 2018 | Fe3O4   | 58.7 nm   | M BALB/c mouse | OVA      | Intravenously     | 1–100 µg            | Attenuation in TH17 responses |
|           | Choi et al. 2011 | SiO2    | 7 nm      | CBA/N mouse | HSEM     | Dermal           | 10–1,000 µg         | SiO2NP did not induce phototoxicity or skin sensitization |
|           | Hira et al. 2015 | SiO2    | 30 nm     | F/NC/Nga slc mouse | HDM     | Dermal           | 20 µL/C2            | Concurrent exposure to allergen and particles resulted in low-level production of allergen-specific IgG subtypes and increased sensitivity to anaphylaxis |
|           | Ostrowski et al. 2014 | SiO2 | 55 ± 6 nm | M SKH1 mouse | Oxazolone | Dermal | –                | Functionalized nanoparticles had no impact on allergic response to oxazolone in an ACD model |
|           | Smulders et al. 2015 | SiO2   | 19 nm     | M BALB/c mouse | DNCB | Dermal           | 0.4, 4.0, or 40 mg/mL x 3 d | SiO2NP exposure prior to sensitization with DNCB did not alter the stimulation index |
| Silver    | Kim et al. 2013 | Ag      | 10 nm     | M SPF guinea pig | GPMT     | Intradermal injection | 0.1 mL 1:1 (v/v) | No eye or skin irritation or corrosion. 1/20 guinea pigs developed erythema following subcutaneous injection, leading to its classification as a weak skin sensitizer |
|           | Bhol et al. 2005 | Ag      | <50 nm    | F BALB/c mouse | DNFb     | Dermal           | 100 mg 1% nanocrystalline | Reductions in ear swelling, erythema, and inflammation were seen after 4 days of treatment with nanoparticle-containing cream |
|           | Smulders et al. 2015 | Ag      | 25–85 nm | M BALB/c mouse | DNCB     | Dermal           | 0.4, 4.0, or 40 mg/mL x 3 d 2% | Exposure prior to sensitization with DNCB did not alter the stimulation index |
|           | Zelga et al. 2016 | Ag      | –        | Guinea pig | GPMT     | Dermal           | – | AgNP-containing dressings for chronic wounds were tested via GPMT, wherein 2/10 animals developed slight erythema that resolved after 72 hours, leading to classification as a mild sensitizer |
|           | Korani et al. 2011 | Ag      | <100 nm   | M Harley Albino guinea pig | –        | Dermal           | 100–10,000 µg/ml    | Dose-dependent increase in number of Langerhans cells recruited to skin |
| Titanium  | Park et al. 2011 | TiO2    | <25 nm    | F CBA/N mouse | LLNA     | Dermal           | 10–1000 µg/mL 50 µg | TiO2 did not induce skin sensitization |
|           | Hussain et al. 2012 | TiO2    | 12 ± 2 nm | M BALB/c mouse | DNCB     | Subcutaneous Injection | 0.004–0.4 mg/mL | TH2 adjuvancy, increased DNCB dermal sensitization potency |

(continued)
Table 2. Continued

| Author(s) | Material | Size | Animal or cell type | Model | Exposure route | Exposure | Dose | Findings |
|-----------|----------|------|---------------------|-------|----------|---------|------|---------|
| Smulders et al. 2015 | TiO₂ | 21 nm | Dunkin-Hartley guinea pig | GPMT | Dermal | 0.1 mL/site | 0.5 g | Increased dermal irritation and sensitization compared to control | ZnO NP did not induce dermal sensitization, acute dermal toxicity, irritation, or corrosion |
| Piasecka-Zelga et al. 2015 | ZnO | 396 nm | New Zealand albino rabbit | Acute | Dermal | 0.5 g | ZnO NP did not induce dermal sensitization, acute dermal toxicity, irritation, or corrosion |
| Dunkin-Hartley guinea pig | GPMT | 0.1 mL/site | 0.5 g | ZnO NP did not induce dermal sensitization, acute dermal toxicity, irritation, or corrosion |

Summary of studies investigating metal nanoparticle immune effects in the skin and select studies using dermal cells, grouped by metal. APS: average particle size, DNCB: dinitrochlorobenzene; GPMT: guinea pig maximization test; HDM: house dust mite; HSEM: Human Skin Equivalent Model; LLNA: Local Lymph Node Assay; OVA: ovalbumin; UV: ultraviolet.

- Dermal exposure to 4.0 mg/mL of TiO₂ prior to sensitization with DNCB resulted in increased stimulation index.
- Zinc-containing nanoparticles caused minor dermal irritation and mild sensitization in hair follicle-associated keratinocyte subpopulations known to have critical roles in the immune response.
- Application of sunscreens containing ZnO NP caused greater increases in blood, urine, and organ Zn ion levels than sunscreens containing larger-sized ZnO particles.
- Although penetration through corneocytes of the stratum corneum is the primary pathway associated with skin penetration by materials, appendages including hair follicles, sebaceous glands, sweat glands, and skin folds can mediate an additional mechanism of skin penetration.
- The diameter of hair follicles can vary greatly in response to anatomical location, but the smallest follicles tend to be located on the forehead and forearm and measure between 66 and 78 μm. Interestingly, the optimal size for penetration of hair follicles is significantly larger than the < 100 nm size range associated with increased skin penetration of several metal nanomaterials. Particles with 600–700 nm
### Table 3. Summary of major findings from studies comparing the effects of various physicochemical properties of metal nanomaterials on dermal allergy grouped by property of interest.

| Property investigated | Author/year | Metal | Study design | Property variations | Findings |
|-----------------------|-------------|-------|--------------|---------------------|----------|
| Size | Kang H et al. 2017 | Ag | RBL-2H3 mast cells | 5, 100 nm AgNP | 5 nm AgNP induced increases in ROS levels, intracellular calcium, and granule release in mast cells in vitro and earlier and more severe lesions in an AD model in vivo |
| Nabeshi et al. 2010 | Si | X5S2 mouse epidermal Langerhans cell, 0.1–1000 µg/mL | 70, 300, 1000 nm SiNP | Cellular uptake and cytotoxicity increased with reductions in particle size |
| Yoshida et al. 2010 | Si | X5S2 mouse epidermal Langerhans cell, 0–100 µg/mL | 70, 300, 1000 nm SiNP | ROS generation by LC was higher following exposure to the smaller amorphous SiNP |
| Hirai et al. 2012b | Si | NC/Nga mouse | 1136 nm SiO₂NP: 33.2 mV, 264 nm SiO₂NP: 25.8 mV, 106 nm SiO₂NP: 24.3 mV, 76 nm SiO₂NP: 19.3 mV, 39 nm SiO₂NP: 14.0 mV | Reduction in size of SiO₂NPs caused enhanced IL-18 and TSLP production, leading to an enhanced systemic Th2 response and aggravation of skin lesions following challenge with house dust mite |
| Ilves et al. 2014 | Zn | BALB/c mice, OVA ACD | 20 nm, 29 mV or 40 mV ZnONP: 66.5 nm SiO₂ nanosphere: 45.7 mV, 184.9 SiO₂ nanosphere: 33.5 mV, 440.0 nm SiO₂ nanosphere: 66.0 mV | ZnO are not dermal sensitizers and do not induce skin irritation irrespective of size and zeta potential, but may induce phototoxicity |
| CRG | Jang et al. 2012 | Zn | CBA/N Mouse LLNA: 25, 50, or 100 µg/mL ZnONP | 20 nm, 29 mV or 40 mV ZnONP | Small negative and neutral-charged nanoparticles exhibited an immunosuppressive effect, whereas positively-charged particles did not. Positively-charged nanoparticles penetrated skin to a lesser extent. Studies also included 100nm TiO₂NP, 20 nm AgNP, and 20 nm AuNP |
| Jatana et al. 2017b | Si | M/F hairless C57BL6 mouse | 32.7 nm SiO₂, nanosphere: 25.4 mV | Positive, neutral, or negatively-charged |
| Schaeublin et al. 2010 | Au | HaCaT human keratinocyte cells | 1.5 nm AuNP | Cell morphology was disrupted by AuNPs of all 3 charges in a dose-dependent manner. Charged AuNPs caused dose-dependent cytotoxicity and mitochondrial stress |
| CRY | Lee et al. 2011 | Si | J774A.1 mouse macrophages: 0–1,000 µg/mL, 1 or 3 d LLNA: F BALB/c, 1 mg/ear x 3 tx | 100 nm spherical: mesoporous SiO₂: 1150 m²/g, colloidal SiO₂: 40 m²/g | Higher surface area caused decreased cytotoxic and apoptotic cell death. Similarly, higher surface area induced lower expression of pro-inflammatory cytokines. Lower surface area Si particles acted as an immunogenic sensitizer in the LLNA |
| Size | Maquieira et al. 2012 | Al | Mice and rabbits | 40, 3000 nm amorphous Al₂O₃, 300 nm crystalline Al₂O₃ | AINP served as both carrier and adjuvant leading to hapten-specific antibody production dependent on size and crystallinity |
| Braydich-Stolle et al. 2009 | Ti | HEL-30 mouse keratinocytes | 100% anatase TiO₂: 6.3, 10, 40, 50, 100 nm | Both size and crystal structure contributed to toxicity in vitro. Smaller size and less agglomeration increased cytotoxicity, 100% anatase TiO₂ particles, regardless of size, induced cell necrosis, whereas the rutile TiO₂ nanoparticles initiated apoptosis through formation of ROS |

(continued)
diameter have been shown to deposit in the deepest depths of hair follicles, suggesting that agglomerates of nanomaterials in this size range are potentially more hazardous than primary particles (Patzelt et al. 2011; Lademann et al. 2015). Furthermore, preferential accumulation in follicles has been observed in hydrophobic and neutrally-charged nanomaterials (Mahmoud et al. 2017).

Although physicochemical properties of metal nanomaterials have been shown in some instances to impact skin penetration, an assortment of host factors can also impact this process. Variations in epidermal thickness, integrity, degree of hydration, and skin pH, all of which may further differ between gender, can greatly influence skin permeability (Sandby-Moller et al. 2003; Senzui et al. 2010; de Matteis et al. 2016). However, the role of disrupted skin barrier integrity is one of the most commonly-examined host factors with applicability to allergic disease since skin permeability can be increased 4–100 times in individuals with skin allergy (Larese Filon et al. 2016).

Scratching to alleviate itching associated with allergic skin lesions leads to mechanical damage to the upper layers of skin. Similar degrees of damage have been shown to increase in vivo penetration of some metal nanomaterials in humans and rodents (Zhang and Monteiro-Riviere 2008; Gopee et al. 2009; Ravichandran et al. 2011; Prow et al. 2012). In vitro simulations using a human skin model called the Franz Method have demonstrated increased capacity for passage through damaged skin by 25 nm AgNP, 6 nm PtNP, 5 nm rhodium nanoparticles (RhNP), 10 nm PdNP, 78 nm NiNP, and 80 nm CoNP (Larese et al. 2009; Larese Filon et al. 2013; Mauro et al. 2015; Crosera et al. 2016; Filon et al. 2016). Contrarily, studies have shown that penetration of various sizes of TiO2 NP and ZnO NP are not increased in skin damaged by chemical irritants, tape-strip- ping, hair removal, or mechanical force (Senzui et al. 2010; de Matteis et al. 2016). However, the role of mechanical force (Senzui et al. 2010; Lin et al. 2011; Miquel-Jeanjean et al. 2012; Crosera et al. 2015; Xie et al. 2015; Leite-Silva et al. 2016).

A few in vivo studies have also investigated effects of skin barrier dysfunction resulting from existing skin allergy on the penetration of metal nanomaterials (Larese Filon et al. 2016). In a mouse model of skin allergy, ZnO NP skin penetration of allergic skin was size-dependently increased, as 240 nm ZnO particles did not penetrate the skin to a similar degree as 20 nm ZnO NP (Ilves et al. 2014). Studies using nonmetal nanomaterials have also demonstrated that penetration of nanomaterials in allergic skin is size-dependent (Try et al. 2016). ZnO NP were also shown to penetrate allergic skin ex vivo using human skin samples (Szikszaei et al. 2011). Contrarily, application of 35 nm ZnO NP to skin of living human subjects with atopic dermatitis did not lead to penetration into viable skin (Lin et al. 2011). Discrepancies between these studies may be reflective of varying exposure durations, as the study reporting penetration involved continuous exposure of up to 2 weeks, compared to the 4-hr exposure wherein no penetration was observed.

Comparatively, equally prolonged exposure to AgNP-containing textiles did not lead to increased skin penetration in individuals suffering from atopic dermatitis compared to control subjects. Sleeves containing silver particles (30–500 nm) were worn by human subjects for 8 h a day for 5 consecutive days, following which levels of AgNP and aggregates in the skin were quantified. Compromised skin barrier was not associated with increases in AgNP skin accumulation; moreover, no differences in urine Ag levels were observed, indicating that atopic dermatitis did not impact the absorption of ions released from the textiles either (Pluut et al. 2015; Bianco et al. 2016).
Table 4. Metal nanomaterials and corresponding physicochemical properties shown to influence immunological processes involved in the development and augmentation of ACD.

| AOP Step                  | Metal nanomaterial effect                                      | Metal          | Properties implicated | Source             |
|---------------------------|----------------------------------------------------------------|----------------|-----------------------|--------------------|
| Sensitization             | Increased potential for nanomaterial penetration of intact skin| Au             | size, crg             | Labouta et al. 2011|
|                           | Increased potential for nanomaterial penetration of damaged skin| Ti             | size, cry             | Monteiro-Riviere et al. 2011|
|                           | Increased release of ions from parent nanomaterials            | Pd             | size                  | Filon et al. 2016  |
|                           | Accumulation of nanomaterial in follicles and skin folds        | Zn             | mod                   | Leite-Silva et al. 2013|
| Molecular initiating event| Increased potential for metal antigen formation                 | Ti             | size                  | Vamanu et al. 2008 |
|                           | Adsorbed protein conformational changes                         | Au             | size                  | Bastus et al. 2009 |
| Cellular response         | Selective uptake by LC                                          | Si             | size                  | Nabeshi et al. 2010|
|                           | Direct activation of DC                                         | Ti             | size, cry, mor        | Schanen et al. 2009|
|                           | Release of DAMPs from skin epithelial cells > activation of DC  | Fe             | size                  | Murray et al. 2013 |
|                           | Release of DAMPs from dermal immune cells > activation of DC    | Si             | size, SA              | Lee et al. 2011    |
|                           | Altered immunogenicity from adsorption of LPS to surface        | Au             | size, mod, hyd        | Li et al. 2017     |
| Organ response            | Depot formation > altered delivery kinetics                     | Si             | agg                   | Hirai et al. 2015  |
|                           | Nanomaterial antigen vehicle > altered delivery kinetics        | Ti             | –                     | Smulders et al. 2015|
|                           | Accumulation in DC endocytic compartments > interference with antigen processing | Si | mod, crg | Shabhazi et al. 2014|
|                           | Enhanced capacity for cross-presentation to CD8+ T-cells        | Fe             | crg                   | Mou et al. 2017    |
|                           | Increased TH1 signaling                                         | Co             | –                     | Cho et al. 2012    |
| Elicitation               | Increased permeability of endothelial cells                     | Al             | –                     | Oesterling et al. 2008|
|                           | Increased effector T-cell recruitment                           | many           | –                     | Lozano-Fernandez et al. 2014|
|                           | Increased number of DC for T-cell activation                    | Si             | mod                   | Li et al. 2016     |
|                           | Increased number of skin macrophages                            | Fe             | –                     | Yun et al. 2015    |
|                           | Increased neutrophil influx to the skin                         | Ti             | –                     | Goncalves, 2011    |
|                           | Increased number of skin mast cells                             | Ag             | size                  | Kang H et al. 2017 |
|                           | Altered T-cell response to mitogens/allergens                   | Pd             | size, sol             | Reale et al. 2011  |
|                           | Increased IgE-independent mast cell degranulation from allergens| many           | size, SA, crg         | Johnson et al. 2017|
| Chronic effects           | Compromised skin repair mechanisms                              | Ag             | –                     | Vieira et al. 2017 |
|                           | Aggravation of allergic lesion severity                          | Si             | size                  | Hirai et al. 2012  |
|                           | Compromised barrier integrity > increased penetration of allergens| Ag             | size, sol             | Koohi et al. 2011  |

Discrepancies in findings regarding the importance of skin barrier integrity on metal nanomaterial skin penetration may be explained by several observations. The diverse degrees of epidermal barrier function disruption between studies represent a potential source of variation. Complete ablation of epidermal function is only observed in response to severe burns and lacerations; likewise, the diverse mechanisms of experimentally-induced disruptions of the stratum corneum should be compared cautiously. In addition, the pathogenesis of atopic dermatitis between experimentally-induced animal models and humans may explain some discordant findings. The severity of lesions between subjects of human studies is also subject to extreme variation, as well. Since chronic skin inflammation can result in epidermal thickening, enhanced barrier function is not uncommon in many skin disorders (Nohynek et al. 2007). Lastly, differences in exposure conditions and duration, test material formulation, and method of penetration assessment can also serve as a source of variation in conclusions between studies. A notable distinction should be made between test materials, since some studies used pristine metal nanomaterials, and others used commercially-available TiO₂ NP/ZnO NP-containing sunscreens. As noted by Gulson et al. (2012), their observations regarding ZnO NP skin penetration may have been subject to modulation by excipients of the commercial sunscreens used in their study. The sunscreen contained isopropyl myristate, a chemical known to enhance the permeability of the skin, as well as EDTA, a chelating agent which may have influenced the release of ions from ZnONP.

In addition to nanomaterial properties and host factors known to influence the capacity for metal nanomaterials to penetrate the skin, environmental factors may also impact this process. One environmental factor with particular relevance to metal nanomaterials and their use in sunscreens is UVR. Although high levels of UV exposure and subsequent sunburn can significantly disrupt epidermal barrier function, low doses of UV exposure are also known to compromise the integrity of the epidermis (Wolf et al. 1993; Holleran et al. 1997; Biniek et al. 2012). Accordingly, several studies have shown that UV exposure prior to topical application of nanomaterials results in greater depth of penetration by ZnO NP, TiO₂ NP, and QD.
Simultaneously, UVR-induced photoactivation of some metal nanomaterials can facilitate their penetration of the skin. In vitro, UVR-induced ROS production by TiO$_2$ NP, QD, and ZnO NP has been associated with DNA damage, lipid peroxidation, and mitochondrial permeability in skin cells (Tiano et al. 2010; Wang et al. 2013; Petersen et al. 2014; Mortensen et al. 2015; Xue et al. 2015, 2016). Subsequent cytotoxicity to dermal fibroblasts, keratinocytes, and melanocytes is another mechanism by which skin barrier integrity can become compromised as a result of UVR. In vivo, UVR-induced photoactivation of TiO$_2$ NP has been associated with increased adherence to the skin, structural rearrangement of the lipid bilayer, and facilitation of large molecule transdermal penetration (Bennett et al. 2012; Turci et al. 2013; Peira et al. 2014; Pal, Alam, Chauhan, et al. 2016; Pal, Alam, Mittal, et al. 2016). Since the degree of ROS produced in response to UVR has been associated with nanoparticle surface area and reactivity, other related properties such as size, degree of agglomeration, and surface modification may also contribute to skin penetration following UVR exposure (Shen B et al. 2006; Jassby et al. 2012; Yin et al. 2012; Xiong et al. 2013).

Although UVR may contribute to adverse effects following dermal exposure to metal nanomaterials by facilitating skin penetration, it may also present a unique concern with respect to allergy. Many signaling pathways and pro-inflammatory mediators involved in sensitization have been associated with UVR-dependent photoactivation of metal nanomaterials (Murray et al. 2013; Rancan et al. 2014). Moreover, UVR is known to modulate the immune status of the skin by a number of mechanisms. For example, UVA and UVB are known to augment costimulatory molecule expression, compromise antigen presentation, and induce apoptosis of LC (Rattis et al. 1998; Seite et al. 2003; Schwarz 2005). Subsequent effects on the immunological fate of metal nanomaterials on the skin have been demonstrated. In a mouse model, significant depletion of LC (≈80%) following UVR exposure increased skin penetration of QD, but resulted in lower levels of metal ion constituents in the lymph nodes (Mortensen et al. 2013).

**Skin sensitization studies**

The skin sensitizing potential of metal nanomaterials has been investigated in a few studies using traditional in vivo approaches. SiO$_2$ NP, ZnO NP, and TiO$_2$ NP have all been incorporated into the Local Lymph Node Assay (LLNA) (Mandervelt et al. 1997; Baskett et al. 1999). Accordingly, it was demonstrated that topical exposure to 100 nm mesoporous and colloidal SiO$_2$ NP, 7 nm SiO$_2$ NP, and ZnO NP were not capable of inducing the 3-fold increase in lymphocyte proliferation associated with classification as a dermal sensitizer (Choi et al. 2010; Lee et al. 2011; Kim et al. 2016). Similarly, topical exposure to 25 nm TiO$_2$ NP did not induce dermal sensitization in multiple studies; however, subcutaneous injection of equal doses resulted in significant increases in lymphocyte proliferation, suggesting that the inability for TiO$_2$ NP to penetrate the skin might be a limiting factor in the potential to induce dermal sensitization (Park et al. 2011; Auttachoat et al. 2014).

The guinea pig maximization test (GPMT) is another in vivo technique used to evaluate dermal sensitization potential that has been employed in the investigation of several metal-based nanomaterials. In one study, five UV-absorbing materials containing SiO$_2$ NP, ZnO NP, and TiO$_2$ NP were assessed. One out of 10 animals exhibited slight erythema following topical exposure to the ZnO NP and TiO$_2$ NP-containing agents, leading to their classifications as mild skin sensitizers (Piasecka-Zelga et al. 2015). In another study, 1 of 20 animals exhibited discrete patchy erythema following intradermal injection with 10 nm AgNP, leading to its classification as a weak skin sensitizer (Kim et al. 2013). Similarly, AgNP were classified as a Grade II (mild sensitizer) after 2 of 10 guinea pigs exhibited lesions 48 h after application of AgNP-containing sterile gauze (Zelga et al. 2016). However, similar AgNP-containing dressings were actually shown to improve the healing of burn wounds in rats over an 18-d period as compared to rats with dressings lacking AgNP, but the study only examined the localized effects (Pannerselvam et al. 2017). The GPMT has also been used to demonstrate that surface-modified FeNP and hydroxyapatite nanoparticles did not induce skin sensitization (Geetha et al. 2013; Mohanan et al. 2014).

The sensitization potential of 5 and 10 nm AgNP was investigated by Hirai et al. (2016) in a mouse model. Mice were injected with AgNP or Ag ions and lipopolysaccharide (LPS) once a week for 4 weeks, and then intradermally challenged. Interestingly, mice administered Ag ions in the sensitization phase did not develop ear swelling following challenge with any form of Ag. Contrarily, AgNP exposure induced sensitization, wherein the smaller AgNP appeared to have stronger sensitizing potential, which was dependent on CD4$^+$ T-cells and IL-17a, but not IFN$\gamma$. Moreover, ear swelling was observed in response to additional sizes of AgNP (50 and 100 nm) and Ag ions, suggesting that the immune response is not nanoparticle-specific. Further examination revealed that 3 nm NiNP was also capable of inducing sensitization in the model, whereas minimally-ionizable 10 nm AuNP and 10 nm SiNP were not (Hirai et al. 2016).

In addition to in vivo approaches to assess skin sensitization, three non-animal alternative assessment methods based on different steps of the skin sensitization AOP are currently validated by the OECD. While metal nanomaterials have not been incorporated into any of the assays, studies with similar cell lines and endpoints have indicated that many metal nano-materials can induce effects similar to those of other skin sensitizers.

The Direct Peptide Reactivity Assay (DRPA) is an in chemico assay based on the requirement for hapten to bind skin proteins to acquire immunogenicity. Accordingly, the molecular imitating event of dermal sensitization is evaluated by quantification of reactivity of an agent towards synthetic lysine and cysteine residues (Gerberick et al. 2004). While some studies have investigated metal nanomaterials and their interactions with proteins and specific amino acids, implications for their capacity to form hapten/carrier complexes are still unclear. However, cysteine has been associated with decreased stability, increased dissolution, and accelerated ion release from metal alloy nanoparticles and AgNP (Hahn et al. 2012; Ravindran et al. 2013; Siriwardana et al. 2015). Moreover, various amino acids have been associated with preferential binding affinities with respect to AuNP size and TiO$_2$ NP surface charge, supporting a role for multiple physicochemical properties in the molecular initiating event of skin sensitization (Liu et al. 2016; Shao and Hall 2016).

The second validated in vitro assay for determination of skin sensitizing potential involves evaluation of the keratinocyte
response to test agents, since they are a source of numerous mediators that facilitate LC migration, antigen presentation, and T-cell activation during sensitization (Kimber and Cumberbatch 1992). Since many of these mediators are released in response to sensitizer-induced activation of the antioxidant/electrophile sensitization pathway Keap1/Nrf2/ARE, its activation is suggestive of potential for the test agent to contribute to the cellular response of the sensitization AOP (Natsch and Emter 2008; 2016; Ramirez et al. 2014). The human keratinocyte cell line associated with this assay, HaCaT, has been frequently used to investigate potential metal nanomaterial effects on the skin in vitro. Correspondingly, PdNP, AuNP, and PtNP have all been shown to activate the Nrf2 pathway in keratinocytes in vitro (Goldstein et al. 2016; Tsuji et al. 2017). Similarly, zinc-containing QD, ZnO NP, and CuO NP have all been shown to alter expression of several specific genes associated with the Nrf2 pathway, including HMOX1 (Rice et al. 2009; Romoser et al. 2011; Lee et al. 2012).

Prior to the establishment of Nrf2 pathway involvement in keratinocyte responses to skin sensitizers, cytokine release by keratinocytes in vitro was evaluated as an indicator of sensitizing potential (Jung et al. 2016; Koppes et al. 2017). Tumor necrosis factor (TNF)-α is a keratinocyte-derived cytokine involved in sensitization and is critically involved in skin sensitization by chromium and nickel (Ilysh et al. 1995; Wang et al. 2007). Dose-dependent TNFα release has been noted following keratinocyte exposure to AgNP, QD, and ZnO NP, indicating high doses may promote LC maturation and dermal sensitization (Samberg et al. 2010; Romoser et al. 2011; Jeong et al. 2013). IL-18 and IL-1β (cytokines critical for LC activity) have also been shown to be increased by QD, SiO2 NP, TiO2 NP, and AgNP (Ryman-Rasmussen et al. 2007; Samberg et al. 2010; Yazidi et al. 2010; Romoser et al. 2011; Hiroike et al. 2013; Zhang and Monteiro-Riviere 2019).

Another mediator involved in skin sensitization that is differentially-released by keratinocytes in response to irritants and sensitizers is IL-17 (Coquette et al. 2003; Koppes et al. 2017). Though it can also be actively secreted after inflammashome activation, IL-17 is an intracellular molecule that functions as an alarmin (Ansel et al. 1988). During programed cell-death, IL-17 remains associated with chromatin and its sequestration prevents effector functions. Contrarily, under necrotic conditions, it is passively released and bioactive. Accordingly, the mechanism of metal nanomaterial-induced keratinocyte cytotoxicity may significantly impact the development of ACD as a result of differential IL-17 release. Although mechanisms associated the preferential induction of necrosis or apoptosis by nanomaterials have yet to be established, some properties have been correlated to these effects (de Stefano et al. 2012; Mohammadinejad et al. 2019). For example, surface charge of 1.5 nm AuNP was demonstrated to be responsible for the mechanism of cell death in HaCaT cells in vitro. Charged AuNP led to disruptions in mitochondrial membrane potential and intracellular calcium levels causing apoptosis, whereas neutral AuNP were associated with necrotic cell death (Schaeublin et al. 2011). Preferential HaCaT apoptosis or necrosis has also been associated with AgNP surface coating and TiO2 NP crystal phase (Braydich-Stolle et al. 2009; Bastos et al. 2016). Collectively, these findings assert that surface chemistry/reactivity of metal nanomaterials may be a critical property in determining whether dermal exposure results in irritation responses or sensitization.

The last validated alternative approach to evaluate skin sensitizing potential involves assessment of the potential for an agent to induce upregulation of activation markers (CD 86 and CD 54) on human APC. However, since the recommended cell lines for these assays (THP-1 and U937) are representative of general DC and not skin-specific DC, these studies will be discussed in the in vitro section of this review, as they may also apply to respiratory sensitization and augmentation of allergy.

Very few studies have been conducted to investigate metal nanomaterial effects specific to LC. However, topical exposure to <100 nm AgNP in guinea pigs was shown to increase the number of LC at the site of exposure in a dose- and time-dependent manner (Korani et al. 2011). This observation is relevant to skin sensitization since the concentration of LC present in the skin has been correlated with increased susceptibility to ACD development. Other in vivo studies confirmed metal nanomaterials including QD are taken up by LC and subsequently transported to lymph nodes (Jatana et al. 2017b). In vitro, associations with LC have been shown to be influenced by SiNP size and surface functionalization (Vogt et al. 2006; Rancan et al. 2012). Smaller SiNP size has also been correlated to increased uptake, ROS production, and cytotoxicity to LC in vitro (Nabeshi et al. 2010; Yoshida et al. 2014).

A specific observation regarding DC that has implications for ACD and dermal sensitization is that some metal nanomaterials can promote DC cross-presentation. Cross-presentation describes uptake of exogenous antigens and their subsequent processing by pathways normally associated with endogenous antigens. As a result, the exogenous antigen is presented by MHC I molecules to CD8+ T-cells, generating the cytotoxic effector cells characteristic of ACD.

Aluminum nanoparticles (AlINP), AuNP, FeNP, and SiNP have all been shown to modify DC antigen cross-presentation capacity (Blank et al. 2011; Li et al. 2011; Hirai et al. 2012; Jimenez-Periáñez et al. 2013; Kang S et al. 2017; Mou et al. 2017; Dong et al. 2018). The mechanism of antigen uptake by DC is known to influence the preferential association of antigens with MHC I or II molecules. Small lipophilic haptens associated with skin sensitization are known to enter APC via passive diffusion and bind cytoplasmic proteins, favoring their processing by endogenous pathways and presentation by MHC I molecules (Rustemeyer et al. 2006). Accordingly, passive diffusion through cell membranes similar to that demonstrated by charged 15 nm AuNP may result in promotion of cross-presentation (Arvizo et al. 2010; Lin et al. 2010; Taylor et al. 2010). Contrarily, receptor-mediated endocytosis of larger antigens has been associated with cross-presentation when uptake occurs by Fc and mannose receptors (Blum et al. 2013). In this regard, the adsorption of macromolecules, including immunoglobulins, to the surface of nanomaterials and physicochemical properties associated with the adsorption of proteins may be critically influential in determining the route of antigen processing.

Another major determinant of antigen association with MHC I or II is persistence inside DC. Antigens resistant to degradation in endosomes are more likely to be processed by MHC I pathways (Lin et al. 2008; Humeniuk et al. 2017). Likewise, metal nanomaterials with physicochemical properties capable of compromising lysosomal acidification (dissolution rate, surface reactivity) may promote cross-presentation (Accapezzato et al. 2005; Savina et al. 2006). Similarly, endosomal escape following uptake by DC can result in binding to cytosolic proteins and subsequent perception as an endogenous antigen (Lin et al. 2008). One major mechanism of endosomal antigen release leading to cross-presentation is oxidative stress and lipid peroxidation, causing antigen leakage from compromised endosome
membranes (Shen et al. 2006; Shahbazi et al. 2014; Dingjan et al. 2016). Oxidative stress induced by CuNPs, FeNPs, and TiO₂ NP have been shown to cause lipid peroxidation, and these metal nanomaterials have also been associated with enhancing DC cross-presentation (Shukla et al. 2011; Napierska et al. 2012; Manke et al. 2013). Metal nanomaterials have also been associated with the induction of autophagy and production of exosomes by DC, both of which have also been associated with antigen cross-presentation (Moron et al. 2004; Chaput et al. 2006; Crotzer and Blum 2009; Li et al. 2011; Shen T et al. 2018).

**Augmentation of existing or developing skin allergy**

Since dermal exposure to metal nanomaterials nearly always occurs simultaneously to other exposures, their potential to augment skin allergy has been investigated using various allergy models. Metal nanomaterial effects on skin allergy have been studied with respect to both T-cell-mediated ACD and IgE-mediated atopic dermatitis. The effects of metal nanomaterials in ACD models have demonstrated findings suggestive of potential effects during both allergic sensitization and elicitation. In one study, subcutaneous exposure to TiO₂ NP 1 hr prior to skin sensitization with dinitrochlorobenzene (DNCB) increased susceptibility of mice to sensitization, as evidenced by a lower concentration of DNCB required to induce sensitization (Hussain et al. 2012; Smulders et al. 2015). The authors noted that although DNCB is known to induce a TH1-dominant response characteristic of ACD, exposure to TiO₂ NP resulted in a TH12-dominant response in the regional lymph nodes. In a similar study, TiO₂ NP were applied topically 1 day prior to sensitization with DNCB, and the same effect on sensitization as observed (Smulders et al. 2015). A diminished TH1 response was observed and TiO₂ NP were detectable in the lymph nodes. Contrarily, SiO₂ NP and AgNP did not induce alterations to DNCB sensitizer potency in the same model.

In another study by (Jatana 2017a), a panel of metal nanomaterials with various physicochemical properties was analyzed for effects on chemical-induced ACD both during sensitization and challenge. When mice were sensitized to dinitrofluorobenzene (DNFB), co-administration of QD did not impact the severity of the challenge response to DNFB, irrespective of particle charge. However, QD administration simultaneous to DNFB challenge did impact the allergic response. Moreover, the effect was dependent on the charge of the materials. The negatively-charged particles suppressed inflammation, whereas the positively-charged materials enhanced ear swelling. The authors confirmed that sensitization to QD did not occur and suggested that variations in skin penetrating capacity of the differently-charged materials was responsible for the observed effects. The conclusions regarding a critical role for nanomaterial size and charge on modulation of ACD elicitation responses is supported by other findings, as well. Suppressive effects on allergic elicitation have also been demonstrated following application of 20 nm SiNP and <50 nm AgNP-containing cream on ACD reactions to DNFB and 2-deoxyurushiol (Jatana 2017a). Contrarily, exposure to positively-charged functionalized 56 nm SiNP did not augment the severity of oxazolone-induced elicitation responses when topically applied for five consecutive days (Ostrowski et al. 2014).

As highlighted by (Jatana 2017a), ACD responses may be subject to modulation as a result of chemical modifications induced by interactions with metal nanomaterials. In their study, the topical application of nanomaterials was subject to removal prior to application of DNFB. As a result, the particle-specific modulation of allergic skin inflammation was not reflective of blocked adduct formation. Although metal nanomaterials exhibit characteristic-ly increased surface reactivity and catalytic potential, their capacity to impact the chemical properties of skin sensitizing chemicals has not been extensively studied. However, a few studies have demonstrated the potential for such effects to impact both ACD sensitization and elicitation. AlNP and AuNP have been shown to act as non-protein carriers of hapten sensitizers capable of facilitating the generation of hapten-specific adaptive immune responses in vivo (Ishii et al. 2008; Maquieira et al. 2012). Similarly, topical application of ointment containing calcium-based nanoparticles has been shown to capture nickel ions by cation exchange, compromising bioavailability and subsequently preventing the elicitation of nickel-specific ACD (Vemula et al. 2011).

In addition to ACD, metal nanomaterial effects on IgE-mediated atopic dermatitis have also been examined. Atopic dermatitis is generally associated with protein allergens, which under normal circumstances are not capable of penetrating the skin (Smith Pease et al. 2002). However, 100 nm ZnO NP and 5 nm AuNP have been shown to enhance skin penetration by albumin and protein drugs (Huang et al. 2010; Shokri and Javar 2015). Likewise, increased permeability of the skin associated with some metal nanomaterials may represent a mechanism by which exposure may increase susceptibility to atopic dermatitis onset.

Simultaneous exposure to TiO₂ NP, AgNP, and SiO₂ NP during sensitization to house dust mite (HDM) in atopic dermatitis models has been associated with an amplification of TH2 responses. This effect was shown to be more pronounced with decreasing size with respect to AgNP and SiO₂ NP, but not for TiO₂ NP (Yanagisawa et al. 2009; Hirai et al. 2012b; Hirai et al. 2015). Exposure to 5 nm AgNP during sensitization was associated with an augmentation of mast cell activity that resulted in more severe skin lesions that appeared earlier than those induced by 100 nm AgNP (Kang H et al. 2017). Decreases in SiO₂ NP size were also associated with enhanced TH12 responses, as evidenced by increased thymic stromal lymphopoietin (TSLP) and IL-18 production (Hirai et al. 2012b). Decreased particle size has been associated with increased aggravation of atopic dermatitis skin inflammation by nonmetal nanoparticles, as well (Yanagisawa et al. 2010).

Metal nanomaterial-induced modulation of allergic inflammation in the challenge phase of atopic dermatitis has also been demonstrated. Topical application of both 240 and 20 nm ZnO NP resulted in diminished localized inflammation. However, the smaller particle was associated with more pronounced suppression of local inflammation, but simultaneous increases in systemic production of IgE (Ilves et al. 2014).

An interesting observation by Hirai et al. (2015) highlights a potentially critical variation between studies that may explain discordant immune effects induced by similar nanomaterials. Distinction between allergy model studies that may contribute to discordant findings. The authors demonstrated that, in their study, exacerbation of allergic sensitization was dependent on co-administration of HDM antigen and SiO₂ NP. When SiO₂ NP agglomerates were administered at a site distal to that of the allergen, the modulation of antibody production was no longer observed. The dependence of physical associations between nanomaterial and antigen on the subsequent adaptive immune response has been similarly demonstrated by FeNP. In multiple studies, intravenous administration of 58 nm FeNP 1 h prior to subcutaneous ovalbumin (OVA) sensitization resulted in decreased levels of IgG1 and IgG2 and suppression of TH1 and...
In this regard, metal nanomaterials of concern for consumers include many of the materials mentioned above, such as ZnO NP, AgNP, TiO$_2$ NP, and SiO$_2$ NP, as a result of their incorporation into construction materials, sunscreen sprays, disinfectants, and cosmetic powders, which upon use can lead to their inhalation. Workers are at risk for inhalation exposure to these and other highly-produced metal-based nanomaterials (Table 1) (Nanomaterials Future Markets 2015). Effects of metal nanomaterials on pulmonary immunity and asthmatic conditions have been extensively studied and summarized in this section. In addition to studies reporting adverse immune effects in workers subject to metal nanomaterial inhalation, animal studies that have generated evidence of potential for respiratory sensitization and augmentation of asthmatic conditions are discussed. Likewise, Table 5 summarizes studies characterizing individual metal nanomaterial effects on pulmonary immunity. Table 6 summarizes studies to examine the effects of metal nanomaterial physicochemical properties on asthma. Table 7 highlights some processes involved in respiratory sensitization and elicitation demonstrated to be subject to modulation by metal nanomaterials.

**Human studies demonstrating pulmonary immune effects of metal nanomaterials**

A 2014 case study best illustrates the concerns associated with the unknown allergic effects of metal nanomaterials. In the report, a chemist who accidentally inhaled NiNP in the workplace subsequently developed clinical symptoms indicative of IgE-mediated respiratory allergy including throat irritation, nasal congestion, facial flushing, and respiratory distress upon future encounters with NiNP. The chemist also developed previously-nonexistent symptoms indicative of T-cell-mediated ACD in response to non-nanoparticulate forms of nickel in her earrings and belt buckles (Journey and Goldman 2014). In addition to reinforcing existing concerns over increased potential for allergic sensitization as a result of decreased size, the case also emphasized additional concerns reflective of the unique mechanisms of metal allergy. The case showed that sensitization via one exposure route may not limit future elicitation reactions to the same tissue; moreover, sensitization by metal ions, irrespective of original parent material size, may result in elicitation reactions following exposure to both nano- and bulk-sized metal materials. Adverse immune effects with implications for allergy have been investigated in human subjects with risk of inhalation exposure to metal nanomaterials in their workplaces. In one study, it was shown that workers of nanomaterial-handling facilities in Taiwan exhibited increased prevalence of sneezing, dry cough, and productive cough compared to workers with no nanomaterial exposures (Liao et al. 2014). Although the workers were employed by facilities handling SiO$_2$ NP, Fe$_2$O$_3$ NP, AuNP, AgNP, and TiO$_2$NP, it is unclear whether the observed respiratory effects were mediated by adaptive immune responses specific to the metals, or nonspecific irritant mechanisms. Interestingly, increased rates of ACD were also observed in the workers of the nanomaterial-handling facilities, but the inciting agents were not determined. As such, it is unknown if exposure to the nanomaterials induced sensitization or caused increased susceptibility to ACD development in workers.

**Knowledge gaps in metal nanomaterial effects on skin allergy**

Knowledge regarding metal nanomaterial effects on skin sensitization is largely limited to TiO$_2$ NP, SiO$_2$ NP, and ZnO NP. Though the selective investigation of these metals is likely reflective of their significance to consumer skin exposures, titanium, silver, and zinc are not historically associated with clinically-significant rates of ACD in the general population. Accordingly, the observation that some of these metals may have increased potential to induce skin sensitization in nanoparticulate form raises additional concerns over the lack of investigations into nanomaterials comprised of metals commonly associated with ACD (nickel, gold, cobalt).

**Metal-based nanomaterials and asthma**

Respiratory exposure to nanomaterials from naturally-occurring and anthropogenic sources has been taking place for centuries; however, the emergence of engineered nanomaterials and their widespread incorporation into consumer goods pose a risk for inhalation exposures to higher doses of materials with diverse chemical compositions and unique properties (Buzea et al. 2007).
| Metal  | Author/year         | Material | Size       | Animal or cell type                  | Model                  | Exposure route   | Dose         | Findings                                                                 |
|--------|---------------------|----------|------------|--------------------------------------|------------------------|------------------|--------------|--------------------------------------------------------------------------|
| Aluminum | Braydich-Stolle et al. 2010 | Al, Al₂O₃ | 48.08 ± 21.0 nm, 32.71 ± 28.3 nm | Human A549 type II pneumocyte, U937 alveolar macrophage | In vitro 3:1 co-culture | 5–500 μg/mL 24h | Exposure impaired bacterial phagocytic function, induction of NfκB pathway |
| Cerium | Park E-J et al. 2009 | CeO₂ | 130 nm | M ICR mouse | Intratracheal Instillation | 50, 100, 200, 400 mg/kg | Differentiation of naïve T-cells and TH1 cytokine production |
| Meldrum et al. 2018 | CeO₂ | <25 nm APS 166.5 nm agglomerates | F BALB/c mouse HDM | Intranasal Instillation | 75 or 750 μg/kg | Repeated exposure to CeO₂ NP in the presence of HDM caused increased lung eosinophils, mast cells, plasma IgE, IL-4, and goblet cell metaplasia |
| Cobalt | Cho et al. 2012 | Co₃O₄ | 18.4 ± 5.0 nm | F Wistar rat | Intratracheal Instillation | 150 cm² SA | Exposure caused pulmonary alveolar proteinosis and TH1/TH17 dominant responses |
| Verstaelen et al. 2014 | CoO | 7.1 nm | BEAS-2B, A549 epithelial cells | In vitro | 1–60 μg/mL | Alterations in expression of genes associated with innate immunity, T-cell activation, and leukocyte adhesion |
| Copper | Cho et al. 2012 | CuO | 23.1 ± 7.2 nm | F Wistar rat | Intratracheal Instillation | 150 cm² SA | Increased AHR, IgE and mucus production |
| Park et al. 2015 | CuO | <50 nm | F BALB/c mouse OVA | Intranasal Instillation | 25, 50, 100 μg/kg | Aggravated pulmonary inflammation, collagen accumulation and expression of progressive fibrosis markers in lungs |
| Lai et al. 2018 | CuO | 46.5 nm | C57BL/6 mouse | Intranasal delivery | 1, 2.5, 5, 10 mg/kg | | |
| Iron | Park et al. 2015 | Fe₂O₃ | 101.3 ± 4.2 nm | M ICR mouse | Intratracheal instillation | 0.5, 1, or 2 mg/kg | TH1-polarized inflammatory response, GM-CSF, MCP-1, and MIP-1 increase, and increased expression of CD80, CD86, and MHC II expression on lung APCs |
| Park et al. 2010c | Fe₂O₃ | 5.3 ± 3.6 nm | M ICR mouse | Intratracheal Instillation | 250, 500, 1,000 μg/kg | Increases in TH1/TH2 cytokines, B cells, and IgE levels |
| Gold | Hussain et al. 2011 | Au | 40 nm | M BALB/c mouse TDI | Aspiration | 40 μL 0.8 mg/kg | 3x AHR increase |
| Baretto et al. 2015 | Au | 6.3 nm | Swiss Webster and A/J mouse | OVA Intranasal Instillation | 6, 60 μg/kg | Inhibited allergen-induced accumulation of inflammatory cells, pro-inflammatory cytokine production. In A/J mice, AuNPs prevented mucus production and AHR |
| Nickel | Cho et al. 2012 | NiO | 5.3 nm | F Wistar rat | Intratracheal Instillation | 150 cm² SA | Exposure caused pulmonary alveolar proteinosis and TH1/TH17 dominant responses |
| Baker et al. 2016 | Ni | 20 nm | M C57BL/6WT or T-bet/-/ mouse | Aspiration | 4 mg/kg | Increased airway remodeling in T-bet knockout mice with susceptibility to TH2 responses |
| Lee et al. 2016 | NiO | 5.3 ± 0.4 nm | F Wistar rat | Intratracheal Instillation | 50, 100, 200 cm² | Acute neutrophilic inflammation, and eosinophils recruited at days 3 and 4 via eotaxin release |
| Chang et al. 2017 | NiO | – | M Wistar rat | Intratracheal Instillation | 0.015–0.24 mg/kg | Alterations in TH1/TH2 balance were indicative of nitrative stress and NfκB activation |
| Platinum | Park et al. 2010b | Pt | 20.9 ± 11.4 nm | M ICR mouse | Intratracheal Instillation | x3 d | Increase in serum IgE, lung TH2 cytokines, and decrease in CD4/8 ratio |
| Onizawa et al. 2009 | Pt | 2 ± 0.4 nm | DBA/2 mouse | Intranasal instillation | | PNP exerted protective effects from cigarette smoke, prevented NfκB activation, and neutrophilic inflammation |
| Silica | Brandenberger et al. 2013 | Si | 90 nm | F BALB/c mouse | OVA Intranasal instillation | 0, 10, 100, 400 μg | Co-exposure during sensitization caused dose-dependent enhancement of OVA-specific IgE, lung eosinophils, mucus cell metaplasia, and TH2/TH17 cytokine production |
| Silver | Park H et al. 2010 | Ag | 6 ± 0.29 nm | F C57BL/6 mouse | OVA Inhalation | 5 x 20 ppm, 40 mg/kg | Decreased AHR, TH2 cytokines, and ROS levels |
| Jang et al. 2012 | Ag | 6.0 ± 0.29 nm | F BALB/c mouse | OVA Inhalation | 20 ppm/40 mg/kg 5x for 24 h | Suppressed mucus production via VEGF signaling alterations |

(continued)
| Metal | Author/year | Material | Size | Animal or cell type | Model | Exposure route | Dose | Findings |
|-------|-------------|----------|------|--------------------|-------|----------------|------|----------|
|       |             |          |      |                    |       |                |      |          |
| Su et al. 2013 | Ag | 33 nm | F BALB/c mouse | OVA | Inhalation | $3.3 \pm 0.7 \text{mg/m}^3$ | $6h/7 \times 7 \text{d}$ | Increased OVA IgE, proteins associated with immune processes were altered |
| Chuang et al. 2013 | Ag | 33 nm | F BALB/c mouse | OVA | Inhalation | $3.3 \text{mg/m}^3$ | $6h/d \times 7 \text{d}$ | Increased Penh, recruitment of neutrophils, lymphocytes, and eosinophils to the airways |
| Xu et al. 2013 | Ag | 141 nm | F BALB/c mouse | OVA | IP Injection | $0.4, 2, 10 \text{mg/kg}$ | Increased OVA-lgG and TH2 responses, local activation and recruitment of leukocytes |
| Titanium | Ahn et al. 2005 | TiO$_2$ | 0.29 μm | M Sprague-Dawley rat | – | Intratracheal Instillation | $4 \text{mg/kg}$ | Increased BAL IL-13 levels, IL-13-producing mast cells, and goblet cell hyperplasia |
| Park et al. 2009 | TiO$_2$ | 20 nm | ICR mouse | – | Intratracheal Instillation | 5, 20, or 50 mg/kg | Increased BAL and serum IgG levels, altered TH1/TH2 cytokines, increased B cell distribution |
| Larsen et al. 2009 | TiO$_2$ | 28 nm | F BALB/c mouse | OVA | IP Injection | 2–250 μg | TH2 adjuvancy, increased IgE, IgG1, and eosinophil levels |
| Rossi et al. 2010 | TiO$_2$ | 10 x 40 nm | F BALC/c/Sca mouse | OVA | Inhalation | 2 h/d, 3 d/w, x4 w @ 10 mg/m$^3$ | Allergic pulmonary inflammation suppressed by TiO$_2$NP |
| Gustafsson et al. 2011 | TiO$_2$ | 21 nm | M Dark Agouti rat | – | Intratracheal instillation | 5 mg/kg | Increased eosinophil, DC numbers in lungs, lymphocytes recruited mostly CD4+, also included CD8+ T-cells, B-cells, and CD25+ T-cells |
| Hussain et al. 2011 | TiO$_2$ | 15 nm | M BALB/c mouse | TDI | Aspiration | 40 μL @ 0.8 mg/kg | 2x increase AHR |
| Scarino et al. 2012 | TiO$_2$ | 5 nm APS, 168/171 nm agg. | M Brown Norway rat | OVA | Inhalation | 9.4 or 15.7 mg/m$^3$ | Significantly decreased lung leukocytes and plasma/BAL IL-4, IL-6, and IFN-γ over OVA controls |
| Jonasson et al. 2013 | TiO$_2$ | 21 nm | F BALB/c mouse | OVA | Inhalation | 32±1 μg | Aggravated allergic response dependent on dose and timing |
| Fu et al. 2014 | TiO$_2$ | 21 nm | M Sprague-Dawley rat | – | Intratracheal instillation | 0.5, 4.0, 32 mg/kg | Depression in lymph nodes, increased T and B-cell proliferation following mitogen stimulation, enhanced NK activity in spleen, increased B-cells in the blood |
| Choi et al. 2014 | TiO$_2$ | P25 | M New Zealand White Rabbit | – | Intratracheal instillation | 10, 50, 250 μg | Dose-dependent eosinophil influx and inflammation in the lung, but not neutrophil or lymphocyte influx |
| Gustafsson et al. 2014 | TiO$_2$ | 21 nm | M Dark Agouti rat, M Brown Norway rat | OVA | Inhalation | 168–159 μg/d x 10 d | Exposure decreased eosinophilia in OVA-sensitized DA and BN rats, but neutrophil/lymphocyte increase in DA rats |
| Mishra et al. 2016 | TiO$_2$ | 4–8 nm | BALB/c mouse | OVA | IP Injection | 200 μg | Augmented AHR, biochemical markers of damage, and induced a mixed TH1/TH2 response |
| Kim et al. 2017 | TiO$_2$ | 75 nm | F BALB/c mouse | OVA | Inhalation | 50 μg/m$^3$ | Exposure exacerbated AHR and inflammation, increases in IL-1, IL-18 |
| Zinc | Roy et al 2014b | ZnO | <50 nm | F BALB/c mouse | OVA | IP Injection | 0.25, 0.5, 1, 3 mg | Administration with OVA caused increased OVA-lgG1, IgG, eosinophil, and mast cell numbers in lungs and spleen |
| Roy et al 2014a | ZnO | <50 nm | F BALB/c mouse | OVA | IP Injection | 1, 2, 4, and 12 mg/mL | Adjuvant effect on OVA allergy by signaling through TLRs and Src kinase leading to inflammatory responses |
| Huang et al. 2015 | ZnO | 181.5 nm low dose | F BALB/c mouse | OVA | Aspiration | 0.1/0.5 mg/kg | Exposure simultaneous to OVA sensitization resulted in eosinophil recruitment and TH2 adjuvancy |

Summary of findings from in vivo studies investigating immune effects of metal nanomaterials in the lung and select in vitro studies in pulmonary cells, grouped by metal. AHR: airway hyperreactivity; APC: antigen-presenting cell; APS: average particle size; DC: dendritic cell; HDM: house dust mite; IP: intraperitoneal; LPS: lipopolysaccharide; MHC: major histocompatibility complex; OVA: ovalbumin; ROS: reactive oxygen species; RSV: respiratory syncytial virus; TLR: Toll-like receptor; WT: wild-type.
Table 6. Summary of major findings from studies comparing the effects of various physicochemical properties of metal nanomaterials on respiratory allergy grouped by property of interest.

| Property investigated | Author/year | Metal | Study design | Property variations | Findings |
|-----------------------|-------------|-------|--------------|---------------------|----------|
| Size                  | de Haar et al. 2006 | Ti    | F BALB/c/cANNCrl mouse, 200 µg intranasal | Fine TiO$_2$: 250 nm, 6.6 m$^2$/g, Ultrafine TiO$_2$: 29.0 nm, 49.8 m$^2$/g | Exposure to equal mass doses of fine and ultrafine TiO$_2$ resulted in increased TH2 cytokines and serum OA-specific IgE and IgG1 only in animals exposed to ultrafine TiO$_2$ |
|                       | Yoshida et al. 2011 | Si    | F BALB/c mouse, Ovalbumin | Amorphous silica 30, 70, 300, or 1000 nm | Smaller particles induced higher levels of OVA-specific IgE, IgG, and IgG1. Splenocytes from mice exposed to the smallest particle produced higher levels of TH2 cytokines than other groups. |
|                       | Liu et al. 2010 | Ti    | Rats, Intratracheal instillation 0.5, 5, or 50 µg/mL | 5 or 200 nm TiO$_2$ | Decreased chemotactic ability, expression of Fc receptors/MHC II by alveolar macrophages. Phagocytic function was increased at low doses and decreased at high doses |
|                       | Chang et al. 2014 | Ti    | M Sprague Dawley rat Intratracheal instillation: x2, x4 w 0.5, 4, 32 mg/kg | 21 nm TiO$_2$NP, 80% anatase, 20% rutile, 1–2 µm TiO$_2$ anatase | Increased macrophage accumulation and alteration of TH1/TH2 status |
|                       | Ban et al. 2013 | Fe    | F BALB/c mouse, Ovalbumin | Submicron Fe$_2$O$_3$: 147 ± 48 nm, 6 m$^2$/g Fe$_2$O$_3$NP: 35 ± 14 nm, 39 m$^2$/g | High and medium doses of both Fe particles caused decreases in eosinophil influx and OVA-specific IgE levels. However, at the low dose, submicron particles had no effect on allergy, whereas nanoparticles had an adjuvant effect on the TH2 response to OVA |
|                       | Rossi et al. 2010 | Ti    | F BALB/c/Sca mouse, Ovalbumin Inhalation: 10 ± 2 mg/m$^3$ x 12 | Rutile TiO$_2$NP: < 5 µm, 2 m$^2$/g Rutile TiO$_2$NP: 10 x 40 nm, 132 m$^2$/g | Allergic pulmonary inflammation was dramatically suppressed in asthmatic mice exposed to either size TiO$_2$. Leukocyte number, cytokines, chemokines, and antibodies were significantly decreased. |
|                       | Park et al. 2015 | Si    | F BALB/c mouse, Ovalbumin Intranasal inoculation | Spherical SiNP: 12.7 m$^2$/g Mesoporous SiNP: 70.6 m$^2$/g PEGylated SiNP: 12.7 m$^2$/g | Sensitive mice exposed to S-SiNP and M-SiNP exhibited elevated AHR over controls. M-SiNPs induced the greatest degree of exacerbation of allergic effects in the OVA model |
|                       | Han et al. 2016 | Si    | F BALB/c mouse | Spherical SiNP: 12.7 m$^2$/g Mesoporous SiNP: 70.6 m$^2$/g, 100.5 nm PEGylated SiNP: 12.7 m$^2$/g, 439.1 nm | APCs preferentially took up cationic AuNPs, causing upregulation of co-stimulatory molecules. Positive AuNPs enhanced OVA-specific CD4+ T-cell stimulation in the lung draining lymph nodes |
|                       | Seydoux et al. 2016 | Au    | F BALB/c mouse AuNP intranasal instillation: 10 µg | 900 nm AuNP: NH$_3$-PVA, 7.2 mV COOH-PVA 8.2 mV | Uncoated SiO$_2$NPs induced proinflammatory and immunomodulatory effects with increases in lung inflammatory cells, TH2 cytokines. Amino and phosphate surface modifications mitigated these effects, whereas PEG coating did not. |
|                       | Marzaioli et al. 2014 | Si    | F BALB/c mouse, Ovalbumin Intranasal inoculation: 10 mg/kg 6x | Amorphous SiO$_2$NP 15 nm: Uncoated 38 mV, PEGylated 26 mV, Phosphate-coated 43 mV, Amino-coated 0 mV | Asthmatic condition increased nanoparticle uptake. Systemic uptake is higher for PEGylated AuNP compared to citrated AuNPs, but both inhibited inflammatory infiltrates and AHR, wherein inhibition was more significant following exposure to citrated AuNPs. |
|                       | Omlor et al. 2017 | Au    | F BALB/c mouse, Ovalbumin Intranasal instillation | 5 nm AuNP, PEGylated or citrated | Surface coating had minimal effects on inflammation in the lungs of rats, but had significant effects on allergic response. |
|                       | Vennemann et al. 2017 | Zr    | F Wistar rat Intratracheal instillation | APTS, TODS, PGA, or acrylic acid coated 9–10 nm ZnO NPs | Ag50-PVP significantly reduced OVA-induced inflammatory infiltrate in sensitized mice. Lung microbiome was altered dependent on coating. |
|                       | Alessandrin et al. 2017 | Ag    | F BALB/c mice, Ovalbumin Intratracheal instillation: 1–50 µg | PVP-coated AgNP: 97 nm, 6.2 m$^2$/g, 7 mV PVP-coated AgNP: 134 nm, 4.5 m$^2$/g, 7 mV Citrate-AgNP: 20 nm, 30 m$^2$/g, 45 mV | Smaller AgNPs increased AHR on d 1, which persisted to d 7 for the citrate AgNPs only. 20 nm AgNP was more pro-inflammatory but little difference between different surface coatings |
|                       | Seiffert et al. 2015 | Ag    | Brown Norway and Sprague-Dawley rats Intratracheal instillation: 0.1 mg/kg | PVP-coated AgNP: 20 or 110 nm Citrate-capped AgNP: 20 or 110 nm | Serum total and OVA IgE, IgG1 increased in mice treated with the uncoated ZnO particle. However, ZnCl$_2$ did not produce similar exacerbations. TiO$_2$ and SiO$_2$ did not affect OVA-IgE or IgG levels. |
|                       | Horie et al. 2015 | Zn    | F CS7BL/6J mouse, Ovalbumin | Rutile TiO$_2$, Al(OH)$_3$ surf: 30-50 nm 37.1 m$^2$/g ZnO: 21 nm, 49.6 m$^2$/g ZnO, SiO$_2$: Surface: 25 nm, unknown SA, ZnCl$_2$ Amorphous SiO$_2$: 7 nm 300 m$^2$/g, 34 nm 80 m$^2$/g | Smaller AgNPs increased AHR on d 1, which persisted to d 7 for the citrate AgNPs only. 20 nm AgNP was more pro-inflammatory but little difference between different surface coatings |
| Property investigated | Author/year | Metal | Study design | Particle size (nm) | Specific surface area (m²/g) | Zeta potential (mV) | Study findings |
|-----------------------|-------------|-------|--------------|-------------------|-----------------------------|-------------------|----------------|
| Non-crystalline SiO₂ particles in both nano and micron size ranges | Vandebriel et al. 2018 | Ti | F BALB/c mouse, ovalbumin | 30 nm rutile or 10 - 25 nm anatase | Soluble CoNP induced eosinophilic inflammation, whereas insoluble Soluble CoNP induced neutrophilic inflammation. | CoO, 20 ± 0.2 nm, 11.46% solubility | CoO, 20 ± 0.2 nm, 11.46% solubility |
| | | Co | Rat, Intratracheal Instillation | Co3O4: 20.2 ± 0.4 nm, 11.46% solubility | CoO: 65.4 ± 2.8 nm, 92.65% solubility | CoO: 65.4 ± 2.8 nm, 92.65% solubility | CoO: 65.4 ± 2.8 nm, 92.65% solubility |
| | | Zn | Wistar rat | ZnONP: 10.7 ± 0.7 nm | Zn2⁺ ions 92.5 mg/mL | Zn2⁺ ions 92.5 mg/mL | Zn2⁺ ions 92.5 mg/mL |

Evidence for increased potential for respiratory sensitization from animal studies

Assessment of respiratory sensitization presents numerous challenges underscored by the absence of validated in vivo, in vitro, or in silico approaches for identification of potential sensitizers. However, biomarkers with proposed utility for in vivo identification of potential respiratory sensitizers following pulmonary exposure include IgE and Th2 cytokines (de Jong et al. 2009; Chary et al. 2018). These markers have not been employed for direct evaluation of respiratory sensitization potential by metal nanomaterials; however, numerous studies have reported increased IgE levels following in vivo pulmonary exposure to TiO₂ NP, PtNP, FeNP, AgNP, and ZnO NP (Park et al. 2009; Park et al. 2010; Cho et al. 2011; Huang et al. 2015; Seiffert et al. 2015). Many of the same nanomaterials have also been associated with increased Th2 cytokine levels (i.e. IL-4, IL-5, IL-13) following pulmonary exposure (Petitbone et al. 2008; Park 2010; Marzaio et al. 2014). Although these findings are suggestive of the potential for metal nanomaterials to induce asthma, the specificity of IgE molecules was not determined in any studies, the capacity for respiratory sensitization remains speculative.

Assessment of respiratory sensitization potential is further complicated by the absence of an AOP specific to the events associated with asthma development. Moreover, discrepancies in some key events involved in asthma inception by LMW and HMW agents indicate the potential requirement for multiple respiratory sensitization AOP. However, many steps are known to be conserved with respect to sensitization of the skin and lungs; knowledge of metal nanomaterial effects on these processes can provide potential insight regarding their potential to cause asthma.

The induction of respiratory sensitization is ultimately dependent on antigen bioavailability. Although it remains unclear whether nano-scale dimensions of metals increase the likelihood for absorption following dermal exposures, the respiratory tract presents a portal of entry known to be
Sensitization

| AOP step | Metal nanomaterial effect | Metal | Properties implicated | Source |
|----------|--------------------------|-------|----------------------|--------|
| Bioavailability | Increased potential for inhalation | many | dustiness | Evans et al. 2003 |
| | Evasion of uptake by pulmonary macrophages | Si | size, crg, mod | Oh et al. 2010 |
| | Evasion of entrapment by pulmonary mucus | ND | size, crg | Murgia et al. 2016 |
| | Prolonged retention in airways | Al | spec, mor | Park et al. 2017 |
| | Direct translocation across lung epithelial tissue to lymphatics | Au | size, crg | Kreyling et al. 2014 |
| Molecular initiating event | Increased potential for metal antigen formation | Ti | size | Vamanu et al. 2008 |
| | Increased protease activity of protein allergens | Au | size, hyd, mod | Li et al. 2017 |
| Cellular response | Adsorption of LPS to nanomaterial surface | Au | size, hyd, mod | Li et al. 2010 |
| | Increased recruitment of DC to lung | Al | mod | Li et al. 2010 |
| | Direct activation of DC | Ti | size, cry | Winter et al. 2011 |
| | Release of DAMPs from immune cells > activation of DC | Zn | size, mor, SA | Hsiao et al. 2011 |
| | Release of DAMPs from epithelial cells > activation of DC | Ag | size, mod, SA, sol | Hamilton et al. 2014 |
| Organ response | Increased CD4+ T-cell presentation efficiency | Ti | size, mor, cry | Schanen et al. 2009 |
| | Increased polarization of CD4+ T-cells to T2 phenotype | Si | size, mod, SA | Vallhov et al. 2012 |
| | Increased number of B-cells | Ti | size | Park et al. 2009 |
| | Alteration in B-cell expansion/maturation | Au | size | Lee et al. 2014 |
| | Increased production of total IgE | Pt | – | Park et al. 2010b |
| | Increased production of allergen-specific IgE | Zn | size, sol, cry | Horie et al. 2015 |

Elicitation

| Organism response-early phase reaction | Increased IgE-dependent mast cell degranulation | Au | size, mod | Huang et al. 2009 |
| | Increased IgE-independent mast cell degranulation | many | size, SA, crg | Johnson et al. 2017 |
| | Increased number of lung mast cells | Ce | – | Meldrum et al. 2018 |
| | Altered mast cell exocytotic function and granule release | Si | SA | Maurer-Jones et al. 2010 |
| | Altered expression of Fc receptors on immune cells | Ti | size | Liu et al. 2010 |
| | Increased endothelial adhesion molecule expression | Al | – | Oesterling et al. 2008 |
| | Increased neutrophil recruitment | Ag | size, sol | Arau et al. 2015 |
| | Increased eosinophil recruitment | Co | sol | Jeong et al. 2015 |
| | Increased lymphocyte recruitment | Zr | mod | Vennemann et al. 2017 |
| | Increased AHR | Ag | size, mod | Seiffert et al. 2015 |
| | Increased airway smooth muscle contractility | Co/Fe | – | Kapilevich et al. 2012 |
| | Mucus cell metaplasia/mucus hypersecretion | Ti | – | Chen et al. 2011 |
| | Chronic effects | Increased epithelial cell proliferation | Zn | sol | Cho et al. 2011 |
| | Increased fibroblast MMP activity extracellular matrix remodeling | Ti | size, cry, mor | Armand et al. 2012 |
| | Myofibroblast accumulation | Cu | – | Lai et al. 2018 |

Adverse Outcome Pathway (AOP) steps involved in the sensitization and elicitation phases of asthma, metal nanomaterials shown to impact individual steps, and physicochemical properties associated with effects are shown. Physicochemical properties of interest include size, metal speciation (spec), agglomeration (agg), surface modification, physicochemical properties may contribute to their persistence in the respiratory tract. Their increasingly susceptible to smaller materials (Mercer et al. 2018). Nano-materials exhibit a characteristically increased level of “dustiness,” a property which describes the propensity for a material to become airborne following disruption (Evans et al. 2013). Accordingly, the potential for aerosolization and inhalation of metal nanoparticles increases with decreasing size, thereby overcoming one of the limiting steps of respiratory sensitization associated with larger-sized metal particles.

Sensitization of the lungs also requires interactions between the sensitizing agent and APC. The respiratory tract is equipped with an expansive repertoire of defense mechanisms that prevent such interactions, but metal nanomaterials have been shown to have increased capacity to circumvent many of these mechanisms, increasing their potential for uptake by DC. In the upper airways, a layer of mucus lining the lumen functions to trap inhaled antigens and facilitate their translocation out of the trachea by the mucociliary escalator (Moldoveanu et al. 2009). Evasion of the ∼5 μm thick mucus layer has been associated with nanomaterial physicochemical properties including size, surface modification, and surface charge (Samet and Cheng 1994; Yang et al. 2008; Liu et al. 2015; Murgia et al. 2016). Generally, hydrophilic, neutrally-charged nanomaterials with smaller diameters have been shown to penetrate mucus to a greater degree than counterparts with opposing properties (Schuster et al. 2013).

In the lower airways, a similar mechanism of antigen neutralization is facilitated by pulmonary surfactant (Chroneos et al. 2010). In addition to optimizing the mechanics of respiration, surfactant contains proteins capable of binding aeroallergens, accelerating their clearance, and preventing their uptake by APC, thereby inhibiting antigen-specific responses (Malhotra et al. 1993; Wang et al. 1996; Hohlfield 2002; Ruge et al. 2011). Two of these proteins, surfactant protein (SP)-A and SP-D, have been shown to bind to various metal nanomaterials leading to accelerated clearance by phagocytic mechanisms (Ruge et al. 2011). Accordingly, nanomaterials with properties that deter binding to surfactant proteins, such as surface charge, may exhibit increased potential for evasion of clearance by this mechanism, increasing potential for interaction with lung DC (Schulze et al. 2011).
clearance may also be compromised as a result of selective cytotoxic effects on pulmonary macrophages and subsequently fewer viable macrophages capable of neutralizing the nanoparticles. Pulmonary macrophage cytotoxicity has been associated with physicochemical properties including morphology, surface charge, and rate of dissolution (Oh et al. 2010; Hamilton et al. 2014; Shim et al. 2017). Metal nanomaterial-induced alterations in phagocytic activity of pulmonary macrophages, as demonstrated by TiO$_2$ NP, ZnO NP, and AINP, may also contribute to evasion of clearance (Wagner et al. 2007; Liu et al. 2010; Liu H et al. 2013). In addition to compromising the clearance capacity of the phagocytic system on a cellular level, nanomaterials are also associated with maximizing the clearance capacity of the collective phagocytic system. Volumetric loading of alveolar macrophages following inhalation of nanomaterials decreases the efficiency of clearance, extending biopersistence, and increasing the potential for interception by DC (Oberdorster et al. 1992; Blank et al. 2017).

Metal nanomaterial cytotoxic effects on pulmonary macrophages may also promote sensitization by additional mechanisms. Since alveolar macrophages are known to antagonize Th2 responses in the lung and downregulate APC functions, cytotoxic effects may disrupt the maintenance of an immunological tolerant state (Tang et al. 2001). Moreover, their depletion leads to significantly increased recruitment of DC and DC precursors to the lungs (Holt et al. 1993; Jakubzick et al. 2006). Numerous metal nanomaterials are also known to trigger the release of alarmins including IL-1β and -1α by alveolar macrophages; in turn, these can activate DC and facilitate sensitization (Braydich-Stolle et al. 2010; Scherbart et al. 2011; Sandberg et al. 2012; Hamilton et al. 2014; Rabolli et al. 2014; Arai et al. 2015).

Similar to their roles in the development of skin allergy, epithelial cells of the respiratory tract are integral in the development of asthma, and their disruption by inhaled materials can have profound influence on the early events of sensitization (Bergamaschi et al. 2006). A major function of airway epithelial cells is to serve as a physical barrier between inhaled agents that deposit in the airway lumen and DC in the epithelium (Hammad and Lambrecht 2015). The importance of barrier integrity in preventing the development of asthma is illustrated by the barrier-disrupting proteolytic activity shared by many aeroallergens with high rates of sensitivity in the population (Kauffman et al. 2006; Lambrecht and Hammad 2012). The frequent observation that metal nanomaterials are capable of inducing cytotoxicity to pulmonary epithelial cells suggests their potential to increase permeability and passage of antigens from the airway lumen to compartments associated with DC.

Airway epithelial cell cytotoxicity has also been associated with the release of alarmins that have potential to promote DC activation and sensitization. Similar to keratinocytes in the skin, the mechanism of cell death can critically influence the nature of the resultant immune response. For example, the necrotic cell death following pulmonary exposure to beryllium results in the release of mediators including cellular DNA, which is recognized as a DAMP by TLR-9, and promotes the unique T$_{H}1$-mediated effects associated with chronic beryllium disease (McKee et al. 2015). NiNP, AgNP, and CoNP have been shown to induce similar necrotic cell death of bronchial and alveolar epithelial cells (Holt et al. 1993; von Garnier et al. 2005; de Haar et al. 2008; Capasso et al. 2014; Ortega et al. 2014). Contrarily, ZnO NP, CuO NP, TiO$_2$ NP, and CrNP have all been associated with induction of apoptotic cell death in pulmonary epithelial cells (Park et al. 2007, 2008; Ahamed et al. 2011; Sun et al. 2012; Senapati et al. 2015). This effect may further influence the development of respiratory allergy since uptake of these cells is a property exclusive to CD103$^+$ DC, a subset of DC associated with cross-presentation and the subsequent induction of CD8$^+$ effector responses (Desch et al. 2011).

**Incorporation of metal nanomaterials into asthma models**

The impact of metal nano-material exposure prior to sensitization has only been addressed by a few studies using asthma models. Aspiration exposure to ZnO NP, TiO$_2$ NP, NiO NP, CuO NP, or SiO$_2$ NP 1 d before inhalation sensitization to OVA was followed by inhalation challenge and subsequent assessment of asthmatic severity. Soluble metal nanomaterials (NiO NP, ZnO NP, and CuO NP) were associated with elevations in OVA-specific IgE, whereas insoluble SiO$_2$ NP and TiO$_2$ NP were not. Subsequent investigations confirmed the importance of metal ion release in the adjuvant effects on sensitization. The increase in OVA-specific IgE production associated with soluble NiO NP was not conserved in response to insoluble NiO microparticles in the same model (Horie et al. 2015). However, ZnCl$_2$ also did not exert the same increase in OVA-specific IgE caused by ZnO NP. As a result, it was concluded that continuous ion release from nanoparticles was required for the induction of the observed effects (Horie et al. 2016). Exposure to residual oil fly ash particles prior to allergen sensitization has also been associated with adjuvant effects attributable to soluble metal constituents (Lambert et al. 2000).

Concurrent exposure to metal nanomaterials during allergen sensitization has been explored extensively in order to evaluate the potential adjuvant effects of metal nanomaterials on asthma development. This concept has been explored with respect to both systemic and respiratory sensitization routes, as well as independent and dependent of allergen challenge. In the absence of allergen challenge, evaluation of sensitization achieved by intraperitoneal injection is limited to assessment by systemic markers, such as antigen-specific IgE and cytokine levels. Accordingly, co-administration of AgNP and ZnO NP with antigen has been associated with elevated levels of allergen-specific IgE, as well as increased levels of T$_{H}2$ cytokines (Matsumura et al. 2010; Xu et al. 2013). As demonstrated with SiO$_2$ NP, enhanced antibody production has been associated with both increasing dose and decreasing particle size (Toda and Yoshino 2016).

Though few studies have correlated metal nanomaterial properties to adjuvant effects on intraperitoneal sensitization independent of allergen challenge, existing findings are conducive with studies using larger metal particles and nonmetal nanoparticles (Naim et al. 1997; Granum 2001b). The impact of the most extensive number of physicochemical properties with respect to adjuvant effects on OVA sensitization use polystyrene nanoparticles (PSP). Nygaard et al. (2004) used PSP ranging from 58 nm to 11.4 µm to evaluate the influence of particle size, mass, surface area, and particle number. Similarly, Granum et al. (2000) used six sizes of spherical PSP to administer doses with constant mass (12.25 mg), size (0.1 µm), particle number (8 x 10$^{8}$), or surface area (1300 cm$^2$). Both studies showed that serum OVA-specific IgE levels best correlated with particle number and surface area (Granum et al. 2001a; Nygaard et al. 2004).

Similar adjuvant effects have been observed following respiratory sensitization and co-exposure to TiO$_2$ NP, SiO$_2$ NP, and ZnO NP (de Haar et al. 2006; Huang et al. 2015). Increases in OVA-specific IgE and T$_{H}2$ cytokine levels were similarly observed in sensitization models of both animals and human subjects.
associated with decreasing size of SiO$_2$ NP (Yoshida et al. 2011). Moreover, SiO$_2$NP surface properties were shown to impact sensitization independent of allergen challenge. Intranasal exposure to three variations of SiO$_2$ NP (spherical, mesoporous, and PEGylated) simultaneous to OVA sensitization exacerbated pathological changes, inflammatory cell influx, and T$_{H2}$ cytokine responses. These effects were specific to the unique surface chemistry of each type of SiO$_2$ NP, but the most severe responses were associated with the nanoparticle with the highest surface area (Han et al. 2016).

The absence of allergen challenge in these studies helps elucidate the direct effects of metal nanomaterials on sensitization processes. However, another approach to evaluate the same effect involves evaluation of allergic parameters collected in response to allergen challenge. Studies utilizing this approach have similarly demonstrated enhanced asthmatic responses in OVA-challenged mice when intraperitoneal sensitization occurred simultaneous to TiO$_2$ NP and ZnO NP (Larsen et al. 2010; Roy et al. 2014a; Roy et al. 2014b; Mishra et al. 2016).

Although the observed effects may reflect residual impacts of metal nanomaterial respiratory exposure during sensitization, similar adjuvant effects on elicitation responses have been observed following respiratory sensitization and simultaneous metal nanomaterial exposure. Simultaneous administration of SiO$_2$NP, CeO$_2$NP, QD, and TiO$_2$NP with allergen during sensitization led to enhanced asthmatic response severity, as measured by antigen-specific antibody levels, inflammatory cell influx, and T$_{H2}$ cytokine levels after challenge (Brandenberger et al. 2013; Meldrum et al. 2018; Vandebriel et al. 2018; Scoville et al. 2019). Studies using similar sensitization procedures and endpoints have also implicated TiO$_2$ NP crystal structure in adjuvant effects on sensitization (de Haar et al. 2006; Vandebriel et al. 2018).

Metal nanomaterial exposure has also been incorporated into the challenge phase of asthma to evaluate potential modulation of asthmatic responses in established asthmatic conditions. Although some metals, including CuO NP, have been exclusively shown to induce significant aggravating effects on elicitation responses, others, including AuNP, appear to exert protective effects on asthmatic responses (Barreto et al. 2015; Park et al. 2016; Omlor et al. 2017). Contrarily, other metal nanomaterials, including TiO$_2$ NP, have been associated with divergent effects on allergen challenge that appear increasingly susceptible to variation during this phase of asthma. Effects have been reported to be differentially induced according to dose, duration of exposure, and endpoints of assessment (Rossi et al. 2010; Hussain et al. 2011; Jonasson et al. 2013; Kim et al. 2017).

Similarly, after OVA sensitization via intraperitoneal injection, AgNP exposure during allergen challenge has been reported to induce various aggravating and attenuating effects on allergic inflammation. Inhalation exposure to 6 nm AgNP was shown in multiple studies to suppress inflammatory cell influx, airway hyper-reactivity (AHR), mucus hypersecretion, and other measures of asthmatic responses (Park, Kim, Jang, et al. 2010; Jang et al. 2012). Contrarily, in another study with very similar exposure conditions, 33 nm AgNP caused increased airway response, inflammatory cell influx, and OVA-IgE levels over control animals (Chuang et al. 2013; Su et al. 2013). The discrepancies between these studies may be attributable to AgNP size difference, as well as potential variations in particle coating, both of which have been associated with different enthalpy effects on asthmatic responses (Alessandrini et al. 2017). Additionally, the first two studies used the T$_{H1}$-dominant C57BL/6 mouse strain, whereas the second used a T$_{H2}$-biased BALB/c strain (Jones et al. 2013).

Strain-specific immune responses following respiratory exposure to metal nanomaterials during allergen challenge have been demonstrated in other studies, as well (Gustafsson et al. 2014). Studies using SiNP and ZrO NP with variations in surface properties demonstrate that when administered during allergen challenge, surface properties of nanomaterials can differentially aggravate allergic inflammation (Marzaïoli et al. 2014; Park, Sohn, et al. 2015; Vennemann et al. 2017). It has been suggested that particles with higher oxidative potential amplify asthmatic inflammation to a greater degree, which would implicate physicochemical properties such as surface modification in these effects (Li et al. 2009).

**Potential mechanisms of asthma augmentation by metal nanomaterials**

Although asthma models have characterized the potential effects of metal nanomaterial exposure on asthmatic processes, many of the underlying mechanisms of these observed effects remain unclear. However, findings from other studies suggest several mechanisms may be associated with the observed effects of metal nanomaterials on the augmentation of asthma.

Respiratory exposure to metal nanomaterials may increase susceptibility to sensitization by aeroallergens by similar mechanisms previously proposed to contribute to their respiratory sensitization potential. Release of alarmins by airway epithelial cells and resident immune cells, disruption of the T$_{H1}$/T$_{H2}$ balance in the lung, and amplification of oxidative stress by metal nanomaterials may also generate adjuvant effects on sensitization. Similarly, FeNP, TiO$_2$ NP, and SiNP have all been shown induce the release of T$_{H2}$ cytokines including IL-33, TSLP, GM-CSF, and IL-25 by airway epithelial cells, which are known to promote DC maturation (Hussain et al. 2009; Val et al. 2009; Mano et al. 2013; Park, Sohn, et al. 2015).

Evidence also suggests metal nanomaterial exposure can modulate inflammatory pheno-types of existing asthmatic conditions. Two major heterogeneous asthma phenotypes differ based on the presence of neutrophil (T$_{H1}$/T$_{H2}$)- or eosinophil (T$_{H2}$)-dominated inflammation (Fahy 2009; Yu and Chen 2018). Particulate and soluble metals are known to differentially impact the nature of existing allergic airway inflammation by skewing this balance (Schneider et al. 2012). Dissolution kinetics also appear influential in this regard, as CoNP, NiNP, ZnO NP, and CuO NP and their corresponding ions have been shown to differentially recruit eosinophils and neutrophils to the lungs of rats after exposure (Cho et al. 2011; Jeong et al. 2015).

Modulation of elicitation response severity by metal nanomaterials may emerge as a result of modulation of mast cell activity. As a major effector cell in IgE-mediated allergic responses, mast cells have been shown to be potent targets of metal nanomaterial-induced adverse effects in vitro (Feltis et al. 2015; Johnson et al. 2017; Alsaleh and Brown 2018). AgNP, CuO NP, SiO$_2$ NP, and TiO$_2$ NP have all been shown to induce IgE-independent mast cell degranulation depending on physicochemical properties including size, surface area, charge, shape, and the presence of adsorbed surface proteins (Marquis et al. 2011; Aldossari et al. 2015; Alsaleh et al. 2016; Johnson et al. 2017). Modulation of IgE-dependent mast cell degranulation has also been demonstrated by some of the same nanomaterials. TiO$_2$ NP, AuNP, CeO$_2$ NP, ZnO NP, and FeNP have been shown to modulate interactions between allergen and surface-bound IgE molecules, interfering with dimerization and subsequent degranulation (Huang et al. 2009; Ortega et al. 2015). Similarly, mast cell
uptake of metal nanomaterials has been associated with modulation of intracellular calcium signaling involved in mast cell degranulation (Amin 2012; Chen et al. 2012). Accordingly, differential ion release by bulk ZnO particles, ZnO NP, and soluble ZnSO₄ has been correlated with the propensity for OVA-sensitized rat mast cells to degranulate when co-exposed with OVA (Yamaki and Yoshino 2009; Feltis et al. 2015).

Furthermore, TiO₂ NP and AuNP have been shown to alter the exocytic kinetics of granule secretion by mast cells (Marquis et al. 2009). The qualitative and quantitative profile of granule contents has also been shown to be subject to modulation by some metal nanomaterials. The number of molecules per granule has been shown to be impacted by SiO₂ NP as a function of porosity and surface area (Maurer-Jones et al. 2010). Variations in vesicle mediator content has been shown to be augmented by AuNP (Marquis et al. 2009). Since the contents of mast cell granules contribute to vascular permeability and inflammatory cell recruitment, these alterations can greatly impact the severity of allergic elicitation (Dudeck et al. 2011; Weber et al. 2015).

Aggravation of existing asthmatic conditions may also involve non-immunological mechanisms, such as metal nanomaterial-induced alterations to normal physiological processes. For example, increased mucus production by epithelial cells is a hallmark symptom of the early and late phase asthmatic response (Erle and Sheppard 2014). The observation that TiO₂NP and CuONP both increased mucin secretion in human epithelial cells suggests potential to exacerbate asthmatic conditions by contributing to obstruction of airways (Chen et al. 2011; Park et al. 2016). Similarly, TiO₂ NP, AuNP, and AgNP have been shown interfere with optimal pulmonary surfactant functioning, which can cause AHR and increased resistance to airflow (Hohlfeld et al. 1999; Hohlfeld 2002; Bakshi et al. 2008; Schlel et al. 2009; Zhang et al. 2018). AHR may also be modulated by metal nanomaterials as a result of alteration of airway smooth muscle (ASM) contractility. ZnO NP, CuO NP, and TiO₂ NP have all been shown to alter human ASM mechanical function in vitro (Berntsen et al. 2010). Similarly, CoFe₂O₄ NP were shown to potentiate both histaminergic and cholinergic ASM contractility in vivo, which has the capacity to exacerbate symptoms of asthma associated with bronchoconstriction (Kapilevich et al. 2012).

Metal nanomaterial exposure may also exacerbate established asthmatic conditions by accelerating the progression of pathological alterations associated with chronic asthmatic conditions. The repetitive induction and resolution of inflammation induced by asthmatic elicitation leads to anatomical alterations referred to as airway remodeling (Fehrenbach et al. 2017). Histological indicators of these alterations have been reported to be exacerbated by various metal nanomaterials including SnNP (Han et al. 2011). Similarly, cellular indicators of accelerated airway remodeling have been implicated in response to many metal nanomaterials. For example, fibroblast accumulation and increased extracellular matrix deposition is a common contributor to airway remodeling and has been observed in response to NiNP, SiO₂ NP, and CeO₂ NP exposure (Warner and Knight 2008; Han et al. 2011; Ma et al. 2012; Armand et al. 2013; Glista-Baker et al. 2014).

Knowledge gaps in metal nanomaterial effects on asthma

Despite the known capacity for many metals to induce IgE-mediated asthma following inhalation, the potential for metal nanomaterials to induce sensitization of the respiratory tract remains completely unknown. Several other interesting aspects of pulmonary immunity have not been widely addressed with respect to metal nanomaterials, and may have relevance to current observations regarding their effects on asthma. The microbiome is known to significantly impact numerous aspects of allergic disorders, and while some metal nanomaterials associated with antimicrobial activity have been shown to alter the pulmonary microbiome, the implications for asthma remain unknown (Alessandrin et al. 2017; Poh et al. 2018). Similarly, the effects of metal nanomaterials on innate lymphoid cells also remain largely unstudied, but should not continue to be neglected, given the importance of this cell type in allergic disorders. Finally, the capacity for metal nanomaterials to disrupt or prevent the development of immunological tolerance has not been explored, and may be influential in both phases of asthmatic conditions.

Effects of metal nanomaterials on immune cells and allergic processes in vitro

In vivo studies have demonstrated the capacity for metal nanomaterials to augment numerous immunological processes that result in functional implications for allergic disease. However, in vitro investigations have helped elucidate some of the underlying mechanisms responsible for in vivo observations. In this section, major findings regarding the role of metal nanomaterial physicochemical properties on molecular and cellular processes with implications for both ACD and asthma are summarized.

Effects on antigen immunogenicity

Many physicochemical properties associated with the molecular and cellular processes that confer antigen immunogenicity are subject to alteration following interactions with constituents of their environment. In this regard, the immunogenicity of metal nanomaterials may be significantly altered as a result of biocorona formation. Following entry into biological media, macromolecules present in the media interact with and adsorb to the surface of nanomaterials within minutes, forming a layer that defines the bio-identity of the nanomaterial (Corbo et al. 2016). The qualitative profile of adsorbed constituents and quantitative strength of association have been shown to be influenced by nanomaterial properties including size, charge, morphology, surface modification, and hydrophilicity, among other properties (Lundqvist et al. 2008; Dobrovolskaia et al. 2014).

Independent of adsorbed constituents’ identities, macromolecules associations with metal nanomaterial surfaces may induce alterations in physicochemical properties associated with their bioactivity (Dobrovolskaia et al. 2009b; Yin et al. 2015). For example, protein adsorption to 25nm FeNP was associated with a 5-fold increase in hydrodynamic size, which can impact a number of biological effects, such as propensity for cellular uptake (Calatayud et al. 2014). Similarly, adsorption of proteins can mask reactive surfaces of metal nanomaterials, attenuating ROS generation, and subsequently inhibiting a major biochemical mechanism involved in the release of alarmins (Ilinskaya and Dobrovolskaia 2016).

Contrarily, surface adsorption of macromolecules may alter the biological activity of metal nanomaterials in a manner that is dependent on the adsorbed constituent profile. Endo-genous proteins, including immunoglobulins, cytokines, and complement proteins are all constituents of the serum and lung lining fluid known to bind metal nanomaterial surfaces (Neagu et al. 2017). The binding of complement protein C₃b and IgG to
The generation of metal antigens in vitro has been shown to be impacted by the unique physicochemical properties of metal nanomaterials. The size-specific increase in surface energy of TiO$_2$ NP was shown to promote associations between the metal and human serum albumin, resulting in increased bioavailability. The altered propensity for inter-actions with host proteins contributed to the observation that titanium (Vamanu et al. 2008). Adsorption of LPS to nanomaterial surfaces has been shown to enhance inflammatory responses to many metal nanomaterials by lung epithelial cells, and various immune cells (Shi et al. 2010; Liu et al. 2012; Bianchi et al. 2017; Li et al. 2017; Ko et al. 2018). The chemical structure of LPS favors its adsorption to hydrophobic, positively-charged metal nanomaterial surfaces, indicating a role for physicochemical properties such as surface modification in the propensity for associations with immunogenic exogenous molecules such as LPS (Gorbet and Sefton 2005; Li et al. 2017).

Although biocorona formation can augment the immunological fate of a nanomaterial, the interactions may also facilitate alterations in immunogenicity of the adsorbed constituents. Deng et al. (2010) showed that functionalized AuNP were capable of binding fibrinogen independent of nanoparticle size, but certain sizes of AuNP induced conformational changes in the protein. Subsequent alterations in protein structure conferred its recognition by the Mac-1 receptor, subsequently activating NF-κB signaling in innate immune cells. Similarly, Bastus et al. (2009) demonstrated that while macrophages did not recognize AuNP or two biomedically-relevant peptides individually, their conjugation facilitated recognition by TLR-4 and the subsequent induction of pro-inflammatory cytokine production. Since these signaling pathways play critical roles in many of the adjuvant effects mentioned in previous sections, interactions between metal nanomaterials and host proteins can generate novel sources of immunogenicity that may promote allergic processes.

The h-CLAT assay has not been employed to evaluate the sensitizing potential of any metal nanomaterials; however, several studies have investigated metal nanomaterial effects on undiffer-entiated THP-1 cells following a 24-h exposure, and reported activation marker expression. Accordingly, up-regulation of CD86 expression was observed following exposure to surface-modified FeNP, SiO$_2$ NP, and mixed-metal alloy nanoparticles (Liu, Y et al. 2013). de Marzi et al. (2017) exposed THP-1 cells to a wide range of SiO$_2$ particle sizes (10–1430 nm); while all particles promoted activation marker expression, the 240 nm SiO$_2$ particles induced the greatest degree of CD80 expression. Similar findings were reported by an investigation that assessed the potential for metal debris released from orthopedic implants to trigger immune activation. Both ~2 μm cobalt-chromium-molybdenum alloy particles and soluble metal ions induced elevations in THP-1 co-stimulatory molecule expression, suggesting that a wide range of metal particle sizes have the capacity to induce immune effects involved in allergic sensitization (Caicedo et al. 2010; de Marzi et al. 2017). Contrarily, no elevations in THP-1 expression of CD86 or CD54 were observed in response to 100 nm AgNP exposure (Gabbiati et al. 2018).

Though CD86 and CD54 are the validated biomarkers indicative of sensitizing potential in the THP-1 line, limited reports have evaluated these specific markers following metal nano-material exposure. However, other markers indicative of DC activation, such as MHC II, CD11b, CD14, CCR2, and CCR5 have been reported to be up-regulated in response to exposures to ZnO NP and FeNP (Prach et al. 2013; Matuszak et al. 2015). Similarly, modulation in expression of 60 genes – several of which were correlated to monocyte differentiation and matur-ation – were observed in response to PtNP exposure (Gatto et al. 2018).

The THP-1 cell line has also been used to identify potential skin sensitizers in vitro based on a unifying property of rapid ROS production following exposure to skin sensitizing chemicals (Miyazawa and Takashima 2012). A similar response has been demonstrated in the cell line following exposure to <100 nm silver-copper alloy nanoparticles, AgNP, CoO NP, PdNP, and NiNP (Monprasit et al. 2018). The degree of ROS production by THP-1 cells has been correlated to properties including particle size and corona presence, as well as exposure dose and duration (Foldbjerg et al. 2009; Casals et al. 2011; Neubauer et al. 2015). Subsequent activation of the p38 MAPK signaling pathway, alterations in expression of HMOX1 and other oxidative stress genes have also been used as in vitro biomarkers suggestive of sensitizing potential. Numerous metal nanomaterials have been associated with these effects on THP-1 cells, which suggests their potential to activate DC and promote sensitization (Mohamed et al. 2011; Khatri et al. 2013; McConnachie et al. 2013; Boonrungsiman et al. 2017).

The potential for metal nanomaterials to induce DC activation has been more extensively examined using primary DC than the cell lines used in the validated assays (Kang and Lim 2012). Although the expression of activation markers in murine bone marrow-derived DC (BMDC) or human monocyte-derived DC (MDDC) has not been validated by OECD for use in determin-ing sensitization potential in vitro, several studies have reported their capacity to accurately predict sensitizers (Tuschl et al. 2000; Pepin et al. 2007). Accordingly, TiO$_2$ NP, ZnO NP, and SiO$_2$ NP have been associated with increased expression of CD80 and CD86 by murine BMDC with respect to size, surface chemistry, and crystallinity (Palomäki et al. 2010; Heng et al. 2011; Winter...
Non-nanomaterial-induced modulated DC activity can also influence sensitization indepen-dent of their capacity to induce phenotypical maturation. For example, uptake of AuNP and AgNP resulted in an enhanced capacity for DC maturation in response to other immune stimuli (Orlowski et al. 2018). This finding suggests uptake of antigens normally incapable of activating DC may trigger their maturation in the presence of nanomaterials. In addition, accumulation of metal nanomaterials has been proposed to interfere with antigen processing and presenta-
tion by DC (Thiele et al. 2003; Humeniuk et al. 2017).

Polarization of DC and the subsequent preferential generation of T_{H1}/T_{H2} effector T-cells is another step in the development of allergy that has been shown to be susceptible to modulation by nanomaterials. Dermal and respiratory sensitizers are associated with divergent oxidative stress responses that induce selective alterations in three major signaling pathways responsible for DC polarization (Mizuashi et al. 2005; Antonios et al. 2009). Polarization of DC towards T_{H1}-promoting activity has been associated with the propensity for skin sensitizers to react with cytoplasmic glutathione following which, rapid depletion leads to ROS accumulation. The rapid induction of oxidative stress induced by contact sensitizers is responsible for the selective activation of the p38 MAPK and JNK signaling pathways within minutes of encounter (Nakahara et al. 2006). Contrarily, polarization of DC towards T_{H2}-dominant responses has been associated with delayed induction of oxidative stress resulting from the preferential association of respiratory sensitizers with intracellular amine groups (Ferreira et al. 2018). Subsequently, selective activation of the NF-κB and ERK pathways occurs.

Knowledge of these pathways and their differential activation explain the observation that metal nanomaterials with opposing catalytic properties induce different polarization profiles in DC in vitro. The oxident capacity of TiO_{2} NP resulted in potenti-ation of DC maturation leading to a T_{H1}-biased responses, whereas treatment with the anti-oxidant surface activity of CeO_{2} NP resulted in secretion of anti-inflammatory IL-10 and a T_{H2}-dominant T-cell profile (Schanen et al. 2013). FeNP, AuNP, and GdNP have also been associated with modulation of DC polarization in vitro with respect to size and surface chemistry (Yang et al. 2010; Vallhov et al. 2012; Tomić et al. 2014; Hoang et al. 2015).

**Effects on processes involved in elicitation of allergy**

Metal nanomaterials have been shown to have potential to influence elicitation reactions specific to both metals and environmental proteins in vitro. With respect to metal allergy, PBMC isolated from women with established allergic sensitivity to palladium were challenged with either 5–10 nm PdNP or palladium salts in vitro (Reale et al. 2011). Variations in TNFα and IL-10 release were noted between exposures, indicating a potential role for metal solubility on metal-specific allergy elicitation. With respect to environmental allergens, basophils isolated from patients with established sensitivity to common environmental allergens including birch pollen, timothy grass pollen, and house dust mite were exposed to AuNP-conjugated with corresponding allergenic proteins. Stable coronas were formed by all three allergens, but binding of allergen to AuNPs caused enhanced activation of basophils in response to house dust mite challenge, as well as birch pollen in some individuals (Radauer-Preiml et al. 2016).

Although lymphocyte cytotoxicity is an immunotoxic effect most often associated with immunosuppression, as major effector cells of both IgE and T-cell-mediated allergic responses, this effect has potential to impact allergic disorders, as well. Accordingly, many metal nano-materials have been shown to be cytoxic and genotoxic to human and murine lymphocytes in vitro. Interestingly, T- and B-lymphocytes have been shown to be more resistant to adverse effects of ZnO NP compared to other immune cell types (Hanley et al. 2009). Although ion release from ZnO NP and PdNP was correlated to cytotoxicity and alteration in gene expression, DNA damage induced by CoNP was shown to be more severe than that induced by Co ions (Jiang et al. 2012; Tuomela et al. 2013; Petrarca et al. 2014; Simon-Vazquez et al. 2016). Susceptibility to cytotoxicity was shown to be reflective of cell cycle status, explaining the finding that memory T-cells were more sensitive to metal nanomaterial effects compared to naïve T-cells (Hanley et al. 2009; Shabbazi et al. 2013).

Modulation of T-lymphocyte activity by metal nanomaterials also has the potential to significantly influence allergic processes. PdNP, AuNP, CoNP, and GdNP have all been shown to induce differential T_{H1}/T_{H2}-biased cytokine production by lymphocytes in vitro dependent on size, solubility, and hydrophobicity (Petrarca et al. 2006; Liu et al. 2009; Boscolo et al. 2010; Moyano et al. 2012). FeNP suppressed the activity of Kv1.3 channels, which suggests a potential mechanism of lymphocyte cell signaling (Yang et al. 2015). Additionally, delays in proliferation, altered mitogen responses, and morphological changes have also been observed by lymphocytes following exposure to various metal nanomaterials (Shin et al. 2007; Beer et al. 2008; Liptrott et al. 2014; Devanabanda et al. 2016). Knowledge regarding effects of metal nanomaterials specifically on B-cells in vitro is limited to AuNP, which have been shown to be size-dependently taken up by B-cells, causing alterations in NF-κB and blimp1/pax5 signaling pathways, and altered secretion of immune-globulins in a size-dependent manner via (Sharma et al. 2013; Lee et al. 2014). The potential for metal nanomaterials to directly alter B-cell processes, such as antigen-specific interactions with T-cells, isotype switching, and affinity maturation, events critical to their effector functions in IgE-mediated allergic disorder such as asthma, remains largely unstudied (Luo et al. 2015).

**Knowledge gaps in metal nanomaterial effects on immune cells and processes in vitro**

Formation of the nanomaterial biocorona has been almost exclu-sively investigated with respect to the adsorption of proteins. However, nanomaterials are also subject to interactions with other macromolecules present in biological fluids, such as nucleic acids and lipids. Adsorption of these molecules may have notable impacts on the immune effects of nanomaterials since different nucleic acids are alarmins recognized by PRR and lipid mediators play critical roles in many aspects of allergic disorders (Schauberger et al. 2016; Muller et al. 2018). Accordingly, more research should be directed towards investigating the biological implications of surface-adsorbed macromolecules other than pro-
tiens. Similarly, it has been suggested that metal nanomaterials may act as soluble or particulate antigens, but the dynamics of metal antigen generation remains largely unstudied with respect to nanoparticles.
**Important considerations for future metal-based nanomaterial allergy studies**

The potential for metal-based nanomaterials to induce immune effects with implications for allergic disease following exposure by routes other than dermal contact or inhalation is a significant knowledge gap. Nanomaterials are being increasingly incorporated into foods, beverages, supplements, and packaging, rendering ingestion exposures an increasing concern (Chaudhry et al. 2008). Ingestion of metal nanomaterials has been associated with altered B-cell distribution, increased levels of IgE and IgG, and splenic toxicity, but the implications of these effects on allergy are unknown (Park et al. 2010a; Kim et al. 2014; Sheng et al. 2014). Similar immune effects have been observed following systemic administration of various metal nanomaterials. Although most current uses for metal nanomaterials are not likely to result in systemic exposures, some nanomaterials with expanding biomedical applications present a concern. The significance of this knowledge gap is demonstrated by the numerous adverse effects in patients administered Feraheme (ferumoxytol), an intravenously-administered iron replacement product containing 17–31 nm colloidal Fe₃O₄NP (Lu et al. 2010). In the 5 years following approval for use by the Food and Drug Administration (FDA) in 2009, 79 anaphylactic reactions were reported, of which 19 were fatal.

As more studies are conducted to advance our understanding of the effects of metal nanomaterials on allergic disease, it should be recognized that accurate assessment is dependent on the evaluation of sample contamination with endotoxin (Dobrovolskaia et al. 2009a). As a result of production and handling in non-sterile conditions, engineered nanomaterials are often carriers of impurities including LPS, a potent immunoadjuvant (Smulders et al. 2012). Accordingly, the presence of endotoxin on surfaces of metal nanomaterials could generate a subsidiary but sufficient amount of immunostimulation required to induce allergic sensitization to the metal itself, or to other allergens. Consequently, exposure to immunologically inert metal nanomaterials contaminated with LPS may lead to misidentification of such agents as sensitizers or adjuvants. Many of the studies published prior to this development do not report the presence or absence of endotoxin in samples, and results should be interpreted with caution.

Another consideration for future studies is that the successful correlation of metal nanomaterial physicochemical properties with mechanisms of toxicity is limited by the accurate assessment and reporting of test material characterization. Although thorough material characterization has become recognized as an indispensable step in nanotoxicity studies, discrepancies in property terminology, evaluation methods, property reporting, and the biological relevance of measured properties complicate comparisons between studies. A notable example of inconsistent property terminology is the tendency for non-discriminate reporting of aggregates and agglomerates of primary particles. The irreversible bonds of aggregates and reversible bonds of agglomerates can impact the effective dose surface area, degree of primary particle dissociation, and other properties that dictate in vivo biological effects (Keene and Tyner 2011; Gualtieri et al. 2012; Sharma et al. 2014). Another property subject to inconsistent reporting is nanomaterial surface area. Differences in the material state and method of assessment can generate results representative of different parameters, including volume-specific, geometric, or specific surface area SSA (van Doren et al. 2011; Wohlleben et al. 2017). Although these metrics are often similarly reported by studies, they have been correlated to notable variations in toxic potential (Sager et al. 2016). The use of multiple assessment methods and disclosure of potential sources of measurement variation between studies will help accurately compare the impact of properties on toxic potential in future studies.

Accurate evaluation of nanomaterial physicochemical properties and their correlation to toxic effects has become increasingly relevant as emerging nanomaterials challenge the efficacy of traditional occupational exposure limits (OEL). The majority of respiratory occupational exposure limits do not discriminate for material size, so as new metal-based nanomaterials emerge, they are subject to the same mass-derived values enforced for other materials of the same elemental composition. This issue is proving problematic as nanotoxicity studies continue to demonstrate that mass may not be the best dose metric for prediction of pulmonary toxicity (Schmid and Stoeger 2016). Moreover, as demonstrated by the studies summarized here, metal nanomaterial properties other than mass have been correlated to immune effects following respiratory exposure. Accordingly, size nonspecific OELs may be ineffective in protecting workers from both nanomaterial-induced pulmonary effects, as well as subsequent immune effects (Schulte et al. 2010). This concern has become increasingly recognized, as NIOSH has recommended size-specific exposure limits for TiO₂. Despite the recommended time-weighted average exposure limits of 2.4 mg/m³ for fine TiO₂ and 0.3 mg/m³ for ultrafine and nanoscale TiO₂, the agency responsible for regulating compliance with its own established limits (i.e. OSHA) has not yet adopted size-specific OEL values for TiO₂ (NIOSH 2011).

**Conclusions**

Although there is a growing amount of toxicological data demonstrating the vast potential for adverse immune effects following exposure to metal nanomaterials, advancements in understanding their interactions with biological systems have allowed for their unique characteristics to be harnessed for beneficial applications, as well. Numerous studies have demonstrated the potential utility of metal nanomaterials for novel vaccine adjuvants, drug delivery vehicles, diagnostic approaches, and immunotherapies. However, to optimize the use of metal-based nanomaterials for these and other advantageous purposes, a more complete understanding of their systemic immune effects, mechanisms of immunomodulation, and capacity to induce allergic sensitization is needed.

**Disclosure statement**

The authors declare no conflicts of interest. The authors alone are responsible for the content of this manuscript.

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

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