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MECHANISMS OF TOXICITY (CJ MATTINGLY AND A PLANCHART, SECTION EDITORS)

The Toxicology of Engineered Nanomaterials in Asthma

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Abstract

Purpose of Review The explosive growth of the nanotechnology industry has necessitated the examination of engineered nanomaterials (ENMs) for their toxicity. The unique properties that make ENMs useful also make them a health risk, and individuals with pre-existing diseases such as asthma are likely more susceptible. This review summarizes the current literature on the ability of ENMs to both exacerbate and directly cause asthma.

Recent Findings Recent studies highlight the ability of metal nanoparticles (NPs) and carbon nanotubes (CNTs) to not only exacerbate pre-existing asthma in animal models but also initiate allergic airway disease directly. CNTs alone are shown to cause airway mucus production, elevated serum IgE levels, and increased T_H2 cytokine levels, all key indicators of asthma.

Summary The ability of ENMs to modulate the immune response in asthma varies depending on their physicochemical properties and exposure timing. CNTs consistently exacerbate asthma, as do Ni and TiO_2 NPs, whereas some NPs like Au attenuate asthma. Evidence is strong that ENMs can contribute to allergic airway disease; however, more work is required to determine their mechanisms, and more epidemiological studies are needed to validate results from animal models.

Keywords Asthma · Allergy · Lung · Nanoparticles · Nanotubes · Nanomaterials

Introduction

Asthma is a chronic inflammatory airway disease of increasing prevalence, which affects roughly 26 million people in the USA and 300 million worldwide [1, 2]. Asthma, in general, is characterized by airway hyperresponsiveness (AHR) as well as inflammation and mucus overproduction, which leads to airway obstruction [3]. Asthma is a highly heterogeneous disease and can result from exposure to a variety of allergens like house dust mites, pollen, or mold, as well as other environmental toxicants such as cigarette smoke, diesel exhaust particles, or ozone [4]. Multiple types of asthma have been identified clinically (i.e., allergic and non-allergic); however, the most common and well studied is allergic asthma [4]. In the classic paradigm of allergic asthma, antigen-presenting cells in the lung, mainly dendritic cells, take up and process inhaled allergens and induce activation of naïve T helper cells to T_H2-type cells which go on to produce a variety of chemokines and cytokines to promote recruitment and activation of other immune cells [3–5]. T_H2 cells produce interleukin (IL)-13 and IL-4 and activate B cells, which produce antigen-specific IgE, which subsequently binds to allergen and Fc receptors on mast cells in the lung, cross-linking them and causing degranulation and release of cytokines, leukotrienes, and histamine, leading to inflammation [3]. In addition to these key players, multiple other cell types in the lung have critical roles in asthma. Innate immune cells like macrophages, eosinophils, and neutrophils have multifaceted roles in promoting the inflammatory environment in airways, facilitating allergen processing, and releasing cytokines [3]. Epithelial cells participate in the inflammatory signaling cascade as well, and fibroblasts residing beneath the epithelium contribute to collagen deposition and fibrosis in chronic asthma [3]. Moreover, smooth muscle cells around airways undergo hypertrophy and hyperplasia which contributes to increased AHR during episodes of bronchospasm [6].

Environmental factors capable of causing asthma exacerbations have been studied extensively and include viral infections and exposure to ambient air pollution [7]. Common components of air pollution like ozone, nitrogen dioxide,

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and particulate matter (PM) have been found to cause airway inflammation and exacerbate asthma [8]. Numerous epidemiological evidence suggests that both fine and ultrafine PM are associated with allergy and asthma, indicating that small particle size (≤ 2.5 and ≤ 0.1 μm, respectively) is an important factor when considering the toxicology of PM [9]. Naturally then, with the advent of nanotechnology, the pulmonary toxicology community has been interested in examining the effects that engineered nanomaterials (ENMs) may have on asthma.

ENMs can be generally defined as purposefully designed materials possessing at least one dimension less than or equal to 100 nm and unique physicochemical characteristics not present in their non-nanoscale counterparts of the same composition [10]. In recent years, the number of different kinds of ENMs has grown exponentially, and ENMs are being used in a wide range of applications including electronics, engineering, and medicine [11–13]. ENMs come in a diverse array of materials and shapes including metal nanoparticles (NPs) (TiO_2, ZnO, Au, NiO), silica NPs, and carbon NPs like fullerenes and carbon nanotubes (CNTs). Metal nanoparticles may be spherical or irregular in shape, and CNTs may be either single- or multi-walled. There are also nanofibers, which are similar to CNTs, but are solid, and may be composed of carbon or metals. Examples of the physical characteristics of different kinds of ENMs are depicted in Fig. 1 [14]. This diversity of ENMs means there are a variety of ways they could interact with biological systems to produce toxicity, and over the past 15 years, there have been numerous studies into how ENMs may cause lung diseases like fibrosis and asthma [15]. The purpose of this review is to concisely summarize the current literature on the toxicology of ENMs in relation to asthma; studies on the uses of ENMs for asthma therapeutics will not be discussed. It will first focus on the way in which different types of ENMs exacerbate pre-existing asthma, and follow by examining how ENMs may be able to initiate asthma directly, in the absence of allergens. These concepts are summarized and illustrated in Table 1 and Fig. 2, respectively. There is a severe lack of human evidence for ENM toxicity, meaning this review will focus primarily on evidence from animal and cell models; however, later, the few relevant human-based studies will be discussed.

ENM-Induced Asthma Exacerbations

TiO_2 Nanoparticles

A number of different asthma models have been utilized to study the effects of TiO_2 NPs on the immune response in asthma. Several studies utilize the common ovalbumin (OVA) mouse model, in which mice are sensitized and then challenged by OVA exposure to produce allergic airway disease [16–20]. One of the earliest TiO_2/OVA studies by Rossi et al. compared nanosized and fine TiO_2 and did not find significant differences in endpoints measured, and surprisingly, exposure to either type of TiO_2 decreased asthma endpoints like eosinophil numbers, airway mucus production, and AHR [20]. This could be due to the timing of TiO_2 NP exposure, because interestingly, two other studies found that the order and timing of OVA and TiO_2 NP exposure affected the observed immune modulation [17, 18]. Both of these studies found that TiO_2/OVA-exposed mice had increased AHR and

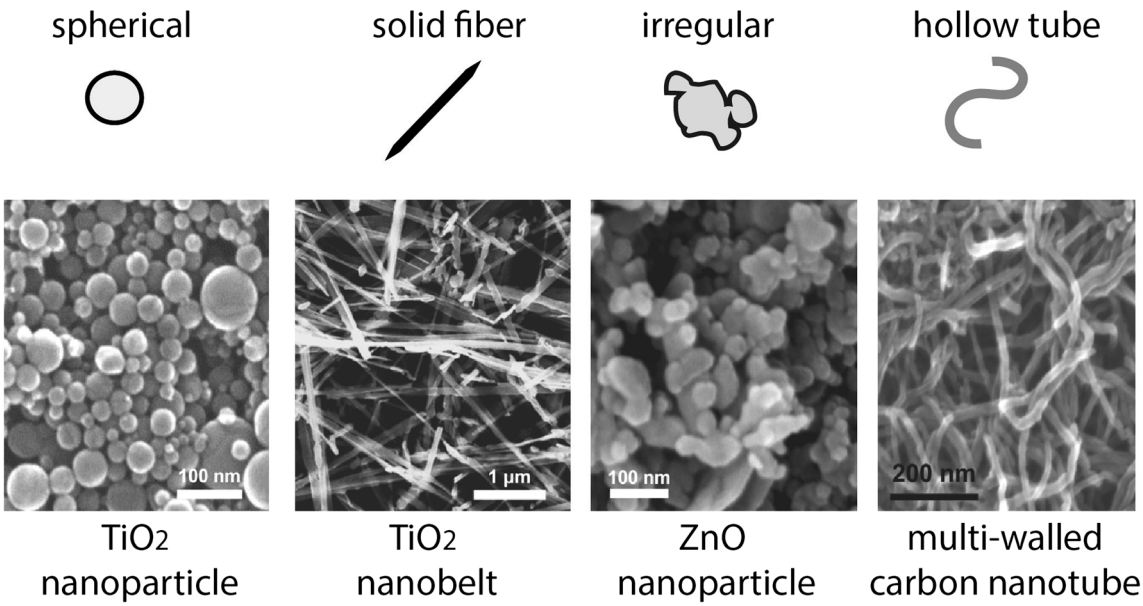

Fig. 1 Examples of different types of engineered nanomaterials with varying shapes. The scanning electron microscope images of the different types of engineered nanomaterials are from Xia et al. [14] and were reproduced from *Environmental Health Perspectives* (https://ehp.niehs.nih.gov/1306561/)

eosinophilia when TiO_2 NPs were given either between the OVA sensitization and challenge phases, or during sensitization, but not when TiO_2 was given during the challenge phase or later, suggesting an adjuvant-like effect [17, 18]. Increases in the T_H2 cytokines IL-4, IL-13, and IL-5 were seen, and Mishra et al. found elevated *Socs3* expression, which is associated with airway inflammation, in TiO_2-exposed mice which was NF-κB dependent [18]. TiO_2 NPs also increase levels of caspase-1 and activate the NLRP3 inflammasome to generate increased production of pro-inflammatory IL-1β, likely through reactive oxygen species (ROS) production [16]. Genetic susceptibility has also been shown to affect the immune response in the OVA model; Gustafsson et al. found differences in TiO_2-induced inflammation in two susceptible rat strains, with both showing exacerbated IgE production and neutrophilia [19]. A study by Hussain et al. utilizing a toluene diisocyanate (TDI) mouse model of asthma similarly found that TiO_2 NPs significantly increased lung inflammation; however, AHR was not increased [21].

Silica Nanoparticles

Several studies have used the OVA rodent model of asthma to examine the toxicity of silica NPs. In rats, SiO_2 NPs administered with OVA were found to increase AHR and disrupt the T_H2-T_H1 balance by increasing IL-4 and decreasing IFN-γ levels in lung protein [26]. Conversely, eosinophil numbers in bronchoalveolar lavage fluid (BALF) were decreased by SiO_2 exposure in this study [26]. The effect of silica NP size on asthma exacerbation was examined by another study, finding that the smaller 30-nm particles were the most bioactive, causing greatly enhanced IgE and IL-4 production when compared to the 70, 300, and 1000-nm particles they tested [27]. Polyethylene glycol-coated (PEGylated) silica NPs have been shown to enhance OVA-induced eosinophilia and neutrophilia, as well as BALF levels of numerous cytokines [28]. Most interesting in this study was the examination of tracheobronchial lymph node cell activation by assessing $CD69^+$ cells by flow cytometry; in particular, alveolar macrophages and dendritic cells were found to have increased activation in silica NP/OVA-treated mice compared to OVA alone [28]. Other studies have examined the differences between spherical, mesoporous (meaning they contain pores between 2 and 50 nm in diameter, giving them a high surface area), and PEGylated silica NPs in OVA mice [29, 30]. Of these, spherical silica NPs were generally the most inflammatory, causing enhanced eosinophilia and AHR compared with OVA alone [29]. In another study by the same group, mesoporous silica NPs were seen to be more inflammatory than spherical; however, both types of silica NPs significantly increased IL-5, IL-13, IFN-γ, and IL-1β over OVA alone [30]. Mesoporous silica NPs could be more inflammatory due to their increased surface area. It should be noted that this study used repeated co-exposure of NPs with OVA and found greater exacerbation than the previous study, which gave NPs only during the challenge phase, indicating again the importance of exposure timing. Silica NPs have been studied in vitro by using spleen-derived antigen-presenting cells to present OVA peptides to T cells, followed by exposure to modified silica NPs [31]. This study found that silica NP exposure enhanced IL-2 and IFN-γ production by $CD8^+$ T cells, indicating the ability of silica NPs to stimulate antigen-specific T cell responses [31].

Ag Nanoparticles

Ag NPs, which are well known for their antimicrobial properties, have been found to modulate inflammatory signaling in asthma models [32]. Ag NPs attenuate OVA-induced allergic inflammation in mice, decreasing total BALF cell counts, IL-4 and IL-13 levels, and *Muc5ac* expression. [32]. This same study also found that Ag NPs decreased VEGF levels and had similar effects in vivo compared to the VEGF inhibitor SU5614, indicating that Ag NP inhibition of VEGF may be at least partly responsible for the attenuated inflammation [32]. Ag NP's ability to attenuate allergic inflammation is supported by another study, which found Ag NPs decreased IL-13, IL-4, IL-5, and NF-κB levels, as well as AHR [33]. Conversely, another study found that Ag NPs increased IgE and IL-13 levels in allergic mice, as well as ROS [34]. However, Ag NPs did not cause any increases in neutrophil or eosinophil BALF numbers, and lung histological changes were not striking [34]. Ag NP effects on OVA-induced allergy were examined by proteomic analysis of BALF and plasma proteins in a study by Su et al. who found a number of proteins induced in Ag NP-exposed mice: apolipoprotein E, myosin light polypeptide 6, and several immunoglobulin components [35]. Even though some of these studies demonstrate suppression of allergic responses, suggesting that Ag NPs may not be deleterious in asthma, it is important to note that Ag NPs have been shown to increase non-allergy-associated pro-inflammatory endpoints like neutrophilia and circulating levels of TNFα, independent of allergen challenge [70, 71].

Au Nanoparticles

Au NPs have been examined in several different ways in relation to asthma. In the study by Hussain et al. discussed above, Au NPs were also used in their TDI-induced asthma model and were found to be even more inflammatory than TiO_2 NPs, showing increased AHR and total BALF cell counts [21]. In the OVA asthma model, Au NPs have been found to actually decrease OVA-induced allergy as seen by decreased inflammatory cell lung accumulation, decreased mucus production, and lower cytokine levels [37]. Similarly, both PEGylated and citrated Au NPs have been shown to

Table 1 Summary of commonly studied asthma endpoints affected by different types of ENMs

ENM	Asthma models	AHR	BALF cell profile	Serum IgE	Cytokines	Direct effects	References
TiO_2	OVA, TDI	↑	↑↑Neutrophils	↑↑	↑IL-1, ↑IL-6, ↑IL-4	↑AHR, ↑mucus, ↑T cell proliferation	[16–25]
Silica	OVA	↑↑	↑↑Eosinophils	↑↑↑	↑↑IL-4, ↓IFN-γ, ↑IL-5, ↑IL-13, ↑IL-1	–	[26–31]
Ag	OVA	↓	↓Total count	↑	↓↓IL-4, ↓IL-13, ↓IL-5	–	[32–36]
Au	OVA, TDI	↓	↓Total counts, ↑eosinophils	No change	–	–	[21, 37–40]
Fe	OVA	–	↓Eosinophils	↓	↓IL-4, ↓IFN-γ	–	[41, 42]
Zn	OVA	–	↑Eosinophils	No change	↑IL-4, ↑IL-13, ↑IL-5	↑eosinophils, ↑IL-4, ↑IL-13	[43]
Cu	OVA	↑	↑Total count	↑	–	↑IL-6, ↑IL-8, ↑Muc5ac	[44–46]
Ni	T-bet$^{-/-}$	–	↑Eosinophils	–	↑CCL2	↑eosinophils, ↑eotaxin	[47, 48]
Ce	–	–	–	–	–	↑IL-4, ↑IL-5	[24]
C	OVA	↑	↑Macrophages	–	↓IL-4, ↓IL-13	–	[49–53]
Polymer	OVA	–	↓Total count, ↓eosinophils	↓	↓IL-4, ↓IL-13	–	[54, 55]
CNT	OVA, HDM, TMA	↑↑	↑↑Eosinophils, ↑neutrophils	↑↑↑	↑IL-4, ↑IL-13, ↑eotaxin, ↑IL-5	↑↑AHR, ↑IgE, ↑mucus, ↑IL-4, ↑IL-13	[56•, 57–66, 67••, 68•, 69•]

Arrows indicate average relative enhancement/suppression

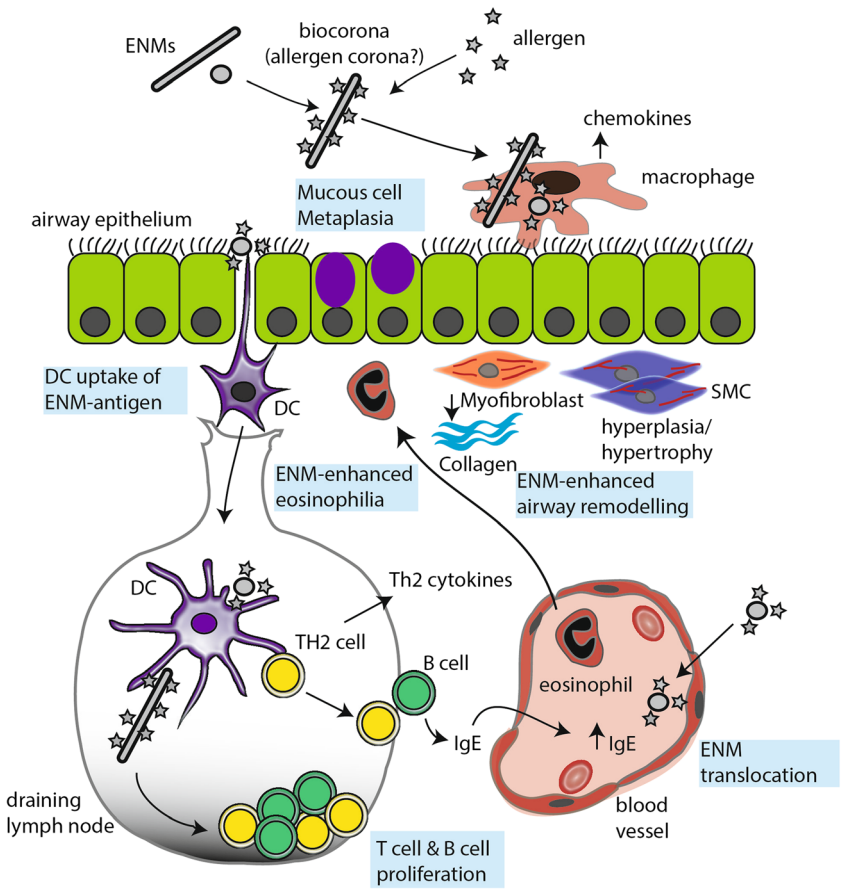

Fig. 2 Illustration of interactions of engineered nanomaterials (ENMs) and allergens with the immune system. ENMs directly stimulate innate immune cells such as macrophages or epithelial cells to produce chemokines that stimulate recruitment of other inflammatory cells such as eosinophils. ENMs also interact with allergens to exacerbate innate immune responses. Dendritic cells transport ENMs to lymph nodes to program T cells as part of the acquired immune system. Phenotypic changes in cells and tissues are shown in *blue boxes*

attenuate OVA-induced inflammation: both types of Au NPs decreased AHR, total BALF cell counts, and eosinophil numbers [38]. This study also examined extrapulmonary uptake of Au NPs, finding that asthmatic mice had more NPs deposited in the spleen [38]. The effects of the protein corona (that is, the proteins that adsorb to the surface of NPs) on Au NP toxicity have been examined by conjugating Au NPs with coronas of common allergens, and Au NPs conjugated with the allergen Der p 1, which is a component of house dust mite, enhanced its protease activity and increased basophil activation in in vitro assays, suggesting that co-exposures of allergens with NPs could enhance inflammation in asthma through corona formation [39]. It has also been found that Au NPs are taken up by eosinophils on the airway surface in OVA-exposed mice [40]. More research will be needed to fully understand the impact of Au NPs on pre-existing asthma as the current studies show somewhat conflicting evidence, with the outcomes depending on the type of asthma model used.

Fe, Zn, Cu, and Ni Nanoparticles

Different sizes of iron oxide NPs have been tested in OVA asthma models and been found to inhibit allergic inflammation, with nanosized particles significantly decreasing eosinophil cell counts and OVA-specific IgE levels, and larger submicron iron oxide particles having no effect [41]. Hematite NPs have been observed to decrease total immune cell numbers in the lungs and lymph nodes of OVA-sensitized mice, an effect which the authors speculate could be due to the acidic nature of the inflammatory environment causing Fe ion release from the nanoparticles, increasing ROS production [42]. Zinc NPs are well known for their toxicity, and ZnO NPs have been tested in the OVA asthma models and were found to increase BALF cell counts and serum IgE levels over OVA alone, an effect that was determined to be Zn ion independent [43]. Copper oxide NPs have also been shown to exacerbate asthma: in an OVA model, they exacerbate numerous endpoints including AHR, inflammatory cell counts, cytokines, IgE, and ROS [44]. This study also found CuO NPs to increase phosphorylation of the MAPKs Erk, JNK, and p38 [44]. Finally, Ni NPs have been found to exacerbate lung inflammation in a transgenic mouse model of asthma susceptibility [47]. This was determined by using mice lacking the T-bet transcription factor, which is involved with T_H1 development, and mice lacking it consequently develop T_H2-type allergic inflammation similar to asthma; this study found that Ni NPs enhanced mucus production in T-bet knockout mice and increased BALF levels of the chemokine CCL2 [47]. Interestingly, through the use of an anti-CCL2 antibody, it was determined that the Ni NP-induced mucus production was at least partly due to these increased CCL2 levels [47].

Carbon Nanoparticles

Carbon black NPs, which are produced through combustion processes, are often used as a control when testing more active types of NPs; however, carbon black NPs themselves have been shown to have the potential to exacerbate asthma [49]. OVA-sensitized mice exposed to 14-nm carbon black NPs had increased numbers of dendritic cells, macrophages, and B cells, as determined by cell surface markers; the larger 56-nm NPs used in this study did not elicit any change from OVA alone, indicating the importance of particle size [49]. These results are supported by another study, which found that carbon black NPs given during OVA sensitization increased inflammatory cell numbers in the lungs as well as $CD8^+$ T cells, $CD4^+$ T cells, and B cells in the lymph nodes [50]. These effects were likely not due to direct particle action on antigen-presenting cells, as in vitro assays on dendritic cells only yielded dendritic cell activation with cell-free BALF from carbon black NP-exposed mice plus carbon black NPs [50]. Carbon black NPs also increase T cell activation in vitro; splenic leukocytes sensitized by OVA peptides with carbon black NPs had enhanced expression of T_H2-associated genes, IL-13, IL-4, and IL-10 [51]. Graphene particles have also been studied in OVA models, yielding contrasting results to carbon black NPs [52, 53]. Graphene oxide was found to decrease markers of T_H2 inflammation like IL-4, IL-13, IL-5, and eosinophils, while increasing airway remodeling and AHR; this increased remodeling may be due to production of chitinases by classically activated macrophages, as macrophages isolated from BALF had elevated acidic mammalian chitinase levels when treated with graphene oxide [52]. In vitro assays with sensitized mast cells and basophils indicate that C_{60} fullerenes inhibit allergic responses, an effect that is in part due to the inhibition of cellular ROS levels [53].

Polymer Nanoparticles

Limited work has been done on asthma and polymer NPs; polystyrene NPs have been examined by two studies [54, 55]. Glycine-coated polystyrene NPs were tested in OVA-induced allergy in mice and were found to inhibit serum IgE, mucus production, and T_H2 cytokines in the lung-draining lymph node [54]. The mechanisms of this inhibition were examined, and polystyrene NPs decreased the numbers of migratory dendritic cells in the lymph nodes, as well as inhibited dendritic cell activation in the lung [54]. Extrapulmonary transport of polystyrene nanoparticles has been examined by using ^{64}Cu-labeled NPs, and the OVA mouse model was used to determine how asthma affects transport [55]. Asthmatic mice had significantly less lung retention of NPs than control mice, and NPs were found in the liver, bladder, and gastrointestinal tract; these results indicate that

asthma may cause a predisposition for greater extrapulmonary toxicity of NPs [55].

Carbon Nanotubes

There is a plethora of literature on the effects of CNTs in mouse models of asthma. Among the first studies to show CNT exacerbation of asthma was a study by Ryman-Rasmussen et al. showing that multi-walled CNTs (MWCNTs) enhanced the development of airway fibrosis in OVA-induced asthma [56•]. IL-13 was not increased by OVA/MWCNTs; however, IL-5 and the profibrotic cytokine PDGF-AA were significantly enhanced by MWCNT, as well as airway collagen thickness [56•]. Several OVA CNT studies share common results: OVA-induced T_H2 inflammation is enhanced over OVA alone by CNT, as seen by increased eosinophilia, serum IgE, and BALF IL-4 levels [57–59]. MWCNTs have also been shown to increase proliferation of OVA-specific T cells in vitro, indicating one possible mechanism of asthma exacerbation [57]. In vitro proliferation and activation of bone marrow-derived dendritic cells has also been observed, demonstrating that antigen-presenting cells may be inappropriately activated resulting in more severe asthma [58]. Many of the studies looking at CNTs fail to measure AHR; however, CNT exacerbation of AHR was observed in two studies [59, 60]. Neutrophilia is enhanced by CNTs in a number of studies, though is less frequent than eosinophilia [56•, 57–59]. CNTs have been found to be more effective at exacerbating allergy than their similar nanosized cousins, carbon nanofibers, as seen by enhanced serum IgE levels; these differences are likely due to CNTs' characteristic of being both longer and thinner than nanofibers [61]. Potential mechanisms of CNT-induced asthma exacerbation have been examined with one study looking at the role of COX-2 [62]. MWCNTs were found to be more effective at exacerbating asthma in COX-2-deficient mice when compared to wild type: IL-13 in particular was greatly increased in the BALF of MWCNT/OVA-exposed COX-$2^{-/-}$ mice, as was serum IgE; interestingly, airway collagen thickness was actually decreased by MWCNTs in this study [62]. The role of STAT1 has also been investigated, and similar to COX-2 deficiency, STAT$1^{-/-}$ mice are more susceptible to asthma exacerbation by MWCNT: mucous cell metaplasia in airways is increased in knockouts, as are TGF-β1, osteopontin, and IL-13, indicating a protective role of STAT1 in asthma [63].

The newer and more clinically relevant house dust mite allergen (HDM) model of asthma has also been used to investigate CNT exacerbation of asthma. As with the OVA model, markers of T_H2 inflammation are increased in HDM/MWCNT-exposed mice over HDM alone: serum IgE, BALF eosinophils, airway mucin, IL-13, and eotaxin [72, 73]. Airway fibrosis is also enhanced by co-exposure to HDM and MWCNTs as seen by lung collagen and higher BALF levels of the profibrotic cytokine TGF-β1 [72, 73]. Interestingly, in one of these studies, it was seen that MWCNTs induced inflammasome activation and subsequent IL-1β production by human THP-1 macrophages in vitro, which was suppressed by the T_H2 cytokines IL-4 and IL-13, which are present in the allergic asthma environment [73]. CNTs have also been looked at in a chemically induced asthma model by using trimellitic anhydride (TMA) to induce allergy in rats [74]. MWCNTs were administered after sensitization and challenge with TMA and, unlike all the above discussed studies, actually decreased TMA-induced serum IgE levels and BALF lymphocytes [74].

ENMs as a Direct Cause of Allergic Airway Disease

TiO_2 Nanoparticles

TiO_2 NPs can elicit several characteristics of allergic asthma directly, both in vivo and in vitro. In rats, a single intratracheal dose of TiO_2 NPs increased BALF eosinophils and neutrophils, the number of airway cells expressing *Muc5ac*, PAS-positive airway cells, and IL-13 expressing cells [22]. In vitro, there is evidence for TiO_2 NP-induced mucus secretion as well: human bronchial epithelial cells treated with TiO_2 NPs show a dose-dependent increase in mucus secretion, which was found to be dependent on Ca^{2+} influx into the cell [23]. Immune cell proliferation and activation is also affected by TiO_2 NPs, which in vitro promote T cell proliferation directly, and TiO_2-treated dendritic cells have an enhanced ability to stimulate $CD4^+$ T cell proliferation [24]. Interestingly, TiO_2 NPs have also been shown to modulate AHR in rats [25]. Rats exposed to TiO_2 NPs by inhalation had increased expression of neurotrophins, which are closely involved with the responsiveness of airway sensory neurons [25]. AHR measurements in these animals mirrored these findings, showing a statistically significant increase with TiO_2 NP exposure [25]. Notable however is that these effects were only seen in newborn and weanling rats, not adults, which could indicate that children are more susceptible to TiO_2 NP-induced airway disease [25].

Zn, Ni, Cu, Ce, and Ag Nanoparticles

Zn, Ni, and Cu NPs are able to recruit eosinophils to the lungs [43, 45, 48]. In epithelial cells in vitro, both Zn and Cu NPs increased IL-8 secretion, and Cu NPs alone increased NF-κB activity; these effects were found to be ion dependent [45]. This study, however, also showed Zn NP recruitment of eosinophils to the lung to be ion independent [45]. Zn NPs also increase BALF levels of the T_H2 cytokines IL-4, IL-13, and IL-5 [43]. In addition to eosinophil recruitment, Cu NPs also induce IL-6, IL-8, and MUC5AC production in bronchial

epithelial cells in vitro, the latter of which is MAPK dependent [46]. Ni NP-induced eosinophil recruitment to the lungs of rats was associated with increased eotaxin, likely released by alveolar macrophages [48]. Ce NPs have been shown to have contrasting effects to TiO_2 NPs in that they act as an antioxidant and in vitro promote a T_H2-type environment when dendritic cells are exposed, which go on to induce naïve T cells to produce IL-4 and IL-5 [24]. Ag NPs have also been shown to promote allergic inflammation in mice when administered with LPS, which was dependent on $CD4^+$ T cells and resulted in increased IL-17A, an effect not seen with Ag ions [36].

Carbon Nanotubes

With CNTs being one of the most heavily studied ENMs in relation to asthma, there is a good deal of evidence that they can elicit allergic lung responses directly. Both single-walled CNTs (SWCNTs) and MWCNTs are able to cause AHR in mice [64, 65]. While a dose-dependent increase was seen in AHR with exposure to SWCNTs, BALF cell counts in these mice did not exhibit the eosinophilia usually associated with allergy, instead showing increased neutrophil numbers [64]. MWCNTs on the other hand exhibited an eosinophilic response in addition to neutrophilia and AHR [65]. The AHR and eosinophilia observed in this study was found to be dependent on both IL-13 and IL-33, but independent of T and B cells, indicating that MWCNTs are modulating the innate immune response to elicit allergy-associated pathologies [65]. Intratracheal instillation of MWCNTs increases the proportion of B cells in the blood, while decreasing natural killer cells and T cells; serum IgE levels are also increased, the level of which rises over time post-exposure [66]. Additionally, IL-1, IL-6, IL-12, and especially IL-10 are significantly increased in the BALF of MWCNT-exposed mice [66]. The physicochemical properties of different types of CNTs have varying effects on the type of immune response they elicit, as examined by two studies [67••, 68•]. Rod-like MWCNTs, which are more rigid, are known to be more toxic than other more flexible MWCNTs and are able to cause AHR, mucus secretion, eosinophilia, and T_H2 cytokine release (IL-4, IL-13) [67••]. These findings are supported by another study, which compared rod-like and the more flexible tangled MWCNTs, finding that rod-like but not tangled MWCNTs induced IL-4, airway mucus production, and elevated serum IgE levels [68•]. In vitro, MWCNTs are able to activate the NLRP3 inflammasome in human bronchial epithelial cells, indicating a potential to cause airway remodeling, a key feature of asthma [69•].

Human Evidence for ENM-Induced Asthma

Though there is no direct human evidence of ENMs causing asthma, the findings of several studies suggest that ENMs may have the potential to cause or exacerbate asthma. A 2014 study used fractional exhaled nitric oxide (FENO) measurement as an endpoint to determine if NPs affect respiratory function [75]. FENO measurement has been recognized as an excellent diagnostic tool for airway inflammation, and FENO is elevated in patients with asthma, atopy, and viral infection [75]. Workers from ENM-handling plants in Taiwan were recruited for the study, and associations were found between the risk level of NP exposure and FENO, with the strongest association being between TiO_2 NPs and FENO [75]. It was also found that asthma and allergic rhinitis were associated with FENO [75]. This study indicates that ENMs, especially TiO_2 NPs, may have the potential to exacerbate asthma. More insight into the effect of NPs on allergic airway disease comes from a case report on a chemist who worked with Ni NPs [76]. Upon initiation of work with Ni NP on an open work bench, the patient experienced throat congestion, flushing of the face, and allergic skin reaction to metal jewelry [76]. The patient received bronchodilators, which improved forced expiratory volume, and the patient also tested positive to nickel in an allergy test [76]. The symptoms exhibited are associated with allergic rhinitis, and the spirometry testing suggests AHR, indicating that Ni NPs can cause allergy, or potentially allergic airway disease [76]. In 2015, a study was published which described CNTs, presumed to be from diesel exhaust, that were found in the airways of asthmatic children [77]. In the study, BALF was obtained from bronchoscopies of asthmatic children, and CNT-like structures were observed inside lung cells. This was intriguing since CNTs can be generated from high-temperature diesel exhaust engines in a laboratory setting [78]. However, no direct evidence was given in the study with asthmatic children that defined the source of the CNT-like structures in inflammatory cells, and therefore, this study lacked a definitive link between exposure and disease outcome. However, it is still worth mentioning because of its real-world relevance for environmental nanomaterial exposure. Future studies should carefully evaluate whether CNTs generated by diesel engines represent a human health risk.

Conclusion

It is clear from the existing studies in rodents that ENMs can modulate the immune response in asthma to either exacerbate or attenuate the disease. ENMs impact a variety of cellular and molecular targets in allergic airway disease including serum IgE, AHR, BALF cell counts, and pro-inflammatory cytokines. ENM modulation of the immune response in asthma is illustrated in Fig. 2. The effects that specific ENMs elicit depend on their, size, shape, composition, and coating. A summary of asthma outcomes that are affected by different types of ENMs can be found in Table 1. Because ENMs are so varied, it will be necessary in the future to continue to

assess their risk to individuals with lung diseases. Strides are being made towards a more mechanistic understanding of ENM-induced allergic lung disease; however, much is still unknown. Additionally, there is a need for more epidemiological studies looking at workplace exposure to ENMs and their respiratory health effects in humans. Along these same lines, it would be advantageous for more studies to look at lower, occupationally relevant doses of ENMs and how exposure affects asthma induced by more relevant common allergens like HDM and cockroach allergens. There is also a need to assess early life exposure, as asthma is more frequent in children [1].

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Declaration of Helsinki and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

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