Published in final edited form as:

J Environ Immunol Toxicol. 2014 ; 1(3): 150–156. doi:10.7178/jeit.23.

Innate Immune Responses to Nanoparticle Exposure in the Lung

Elizabeth A. Thompson, Brian C. Sayers, Ellen E. Glista-Baker, Kelly A. Shipkowski, Alexia J. Taylor, and James C. Bonner

Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, North Carolina, USA

Abstract

The nanotechnology revolution offers enormous societal and economic benefits for innovation in the fields of engineering, electronics, and medicine. Nevertheless, evidence from rodent studies show that biopersistent engineered nanomaterials (ENMs) stimulate immune, inflammatory, and fibroproliferative responses in the lung, suggesting possible risks for lung diseases or systemic immune disorders as a consequence of occupational, environmental, or consumer exposure. Due to their nanoscale dimensions and increased surface area per unit mass, ENMs have a much greater potential to reach the distal regions of the lung and generate ROS. High aspect ratio ENMs (e.g., nanotubes, nanofibers) activate inflammasomes in macrophages, triggering IL-1 β release and neutrophilic infiltration into the lungs. Moreover, some ENMs alter allergen-induced eosinophilic inflammation by immunostimulation, immunosuppression, or modulating the balance between Th1, Th2, and Th17 cells, thereby influencing the nature of the inflammatory response. ENMs also migrate from the lungs across epithelial, endothelial, or mesothelial barriers to stimulate or suppress systemic immune responses.

Keywords

innate immunity; lung; nanoparticles; asthma

Introduction

Nanoparticles have at least one dimension less than 100 nm and due to their nanoscale size have the capability of reaching the distal regions of the lung after inhalation exposure. Engineered nanomaterials (ENMs) are intentionally designed nanoparticles, as opposed to naturally occurring nanoparticles generated by forest fires or volcanic activity; or nanoparticles generated from anthropomorphic activities such as the burning of fossil fuels. The unique properties of ENMs make them ideal candidates for the development of novel treatment strategies for lung and other diseases. However, many of the same unique properties allowing ENMs promise as novel therapeutics also pose potential risks. In fact, a variety of ENMs pose possible risks for lung diseases that could be associated with occupational or consumer exposure.^{1,2} ENMs produced in the highest volume include

carbon nanotubes (CNTs), titanium dioxide (TiO₂), cerium oxide (CeO₂), zinc oxide (ZnO), and silver (Ag) and thus present the greatest likelihood for human exposure. However, risk to human health is not entirely linked to production volume and probability of exposure, but also to reactivity and potency of impact on biological systems, including the immune system. Therefore, ENMs produced in lower quantities that have potent effects on the immune system, such as nickel (Ni) and cobalt (Co) nanoparticles, could present significant health risks.

Immunotoxicity is defined as any adverse effect on the immune system following toxicant exposure that results in immune stimulation or immune suppression.³ Immunostimulation increases the incidence of allergic reactions, inflammatory responses, or autoimmunity, while immunosuppression suppresses the maturation and proliferation of immune cells, resulting in increased susceptibility to infectious diseases or tumor growth. Dependent largely on the specific type of ENM in question, they have been reported as either immunostimulatory or immunosuppressive in the lung. However, the effects of ENMs on the immune system can also depend on the context of exposure; for example, repeated ENM exposures versus ENM exposure after the establishment of allergic inflammation.

The aim of this review is primarily to summarize some of the acute and chronic innate immune responses in the lung induced by select ENMs that could occur from environmental or occupational exposure. While only touching upon the utilization of ENMs for drug or vaccine delivery, careful consideration should be given to the potential risks of ENM-based delivery systems in order to ensure that therapeutic benefits outweigh the possibility of adverse side effects such as immunotoxicity.⁴

Lung deposition and translocation of ENMs

The deposition of inhaled ENMs is determined by a number of factors including particle size, shape, electrostatic charge, and aggregation state. For example, inhalation exposure to well-dispersed CNTs in mice results in deposition in the distal regions; i.e., alveolar duct bifurcations and alveolar epithelial surfaces, of the lungs of mice or rats.⁵⁻⁷ Aggregation of ENMs, alternatively referred to as state of dispersion, refers to nanoparticles that loosely adhere to one another through non-covalent interactions, such as electrostatic charge. Dispersion of aggregated ENMs can be achieved through surface functionalization to reduce electrostatic charge, or by suspension of ENMs in surfactant-containing media. The relative state of dispersion often influences the type of immune or pathologic response to ENMs. For example, dispersed CNTs cause diffuse interstitial fibrosis throughout the lower lung, whereas aggregated CNTs tend to cause focal granuloma formation.⁸ Aggregation of ENMs also depends, to some extent, on the method of delivery to the lungs. Intratracheal instillation or oropharyngeal aspiration techniques for delivery to the lungs of rats or mice can result in more aggregation of ENMs and generally do not faithfully reproduce deposition patterns that are achieved with inhalation exposures to dry aerosolized or nebulized suspensions of ENMs. However, major advances have been made in methods for dispersing ENMs in aqueous suspension using surfactant-containing media prior to instillation or aspiration in rats or mice.⁸ Inhaled or well-dispersed instilled ENMs also reach the sub-pleural region of the lungs either via macrophage-dependent or -independent processes.

Persistent ENMs, such as CNTs, remain embedded within the subpleural tissue of mice for months.⁹ Additionally, some CNT-bearing macrophages can exit the lung via the pleural lymphatic system into the pleural space or can be found in lung-associated lymph nodes.^{5,6}

Effects of ENMs on the innate immune responses of macrophages

Lung macrophages serve a central immune defense role against inhaled particles and fibers. Most inhaled ENMs deposited in the distal regions of the lung are avidly taken up by alveolar macrophages in an aggregated form (clusters of nanoparticles). While aggregated ENMs are engulfed by macrophages, individual nanoparticles can escape immune surveillance and phagocytosis. For example, individual CNTs evade phagocytosis or uptake by macrophages and can be detected by TEM within epithelial or mesenchymal cells.⁹ Aggregated ENMs taken up by macrophages via phagocytosis are cleared from the lungs through two primary mechanisms; 1) the mucociliary escalator and 2) the lymphatic drainage system. The mucociliary escalator is comprised of a coating of mucus on the surface of the airways that is constantly moving up the airways by the coordinated movement of cilia on the airway epithelium.¹⁰ Macrophages with engulfed particles or fibers migrate to the distal portion of small airways where they are transported by the escalator to larger airways and ultimately out of the trachea where they are swallowed or expelled through coughing. A secondary macrophage-mediated clearance passage for ENMs out of the lung is the lymphatic drainage system, which includes lymphatic vessels that drain into the pleural cavity. Rigid, high aspect ratio ENMs (fiber- or tube-shaped) can present a problem for macrophage-mediated clearance if the nanofiber or nanotube exceeds the width of the engulfing phagocyte. For example, migration of macrophages containing CNTs across the pleura could cause DNA damage to mesothelial cells similar to asbestos fibers.^{2,11} However, whether CNTs possess pleural carcinogenicity like asbestos fibers remains unknown. Similarly, inhaled multi-walled CNTs can also reach lung-associated lymph nodes in rats from trafficking of ENM laden macrophages.⁶

Certain high aspect ratio ENMs (rigid CNTs, nanowires, nanofibers) are capable of disrupting macrophage function by causing frustrated phagocytosis, which results in the release of inflammatory mediators (ROS and cytokines) and cell death.^{11,12} The innate immune function of macrophages could also be compromised by the formation of bridges composed of parallel bundles of CNTs that link two or more macrophages.¹³ Macrophage phagocytosis and chemotaxis in rat alveolar macrophages *in vivo* is impaired by exposure to TiO₂ nanoparticles.¹⁴ Additionally, ENMs have also been reported to impair phagocytosis of microbes as seen in mice exposed to single-walled CNTs that have impaired clearance of the bacteria *Listeria monocytogenes*.¹⁵ Furthermore, mitochondrial stress induced by ENMs is yet another cause of macrophage cell death. For example, CeO₂ nanoparticle toxicity to human peripheral blood monocytes was found to be caused by mitochondrial damage and overexpression of apoptosis-inducing factor, but was independent of ROS production, and resulted in autophagy.¹⁶ Autophagy induced by CeO₂ nanoparticles was further increased after pharmacological inhibition of tumor suppressor protein p53. However, inhibition of autophagy partially reversed cell death by CeO₂ nanoparticles.

Inflammasomes activation by ENMs and alternative macrophages

The uptake of ENMs could have a variety of consequences related to macrophage biology and function. The high aspect ratio ENMs (e.g., carbon nanotubes) cause inflammasome activation in macrophages which results in the processing and secretion of the pro-inflammatory cytokines IL-1 β and IL-18 that is mediated by lysosomal disruption and ROS production.¹⁷⁻¹⁹ Moreover, the dysregulation of inflammasomes has been implicated in a variety of disease states.²⁰ Inflammasome activation leading to the release of mature IL-1 β has been proposed as a pro-fibrogenic event²¹ and downstream targets of IL-1R activation include up-regulation for growth factor receptors such as PDGF-R α .²² However, macrophage IL-1 β release is also an important innate immune response for recruiting neutrophils to the lung to participate in microbial killing and the resolution of inflammation.²³ As illustrated in Fig. 1, exposure to certain ENMs (TiO₂, ZnO, CNTs) results in a Th1 immune cell microenvironment that promotes polarization of “classically activated macrophages” (CAMs).²⁴ CAMs are capable of inflammasome activation and are thought to play essential roles in microbial killing and innate immune responses. In allergic asthma or fibrosis, macrophages are polarized to “alternatively activated macrophages” (AAMs) in the presence of Th2 cytokines, IL-4 or IL-13, and are thought to play important roles in fibrosis and cancer.²⁴ While little is known about inflammasome activation and IL-1 β release by AAMs, compared with CAMs, a decrease in caspase-1 expression and pro-IL-1 β processing has been described for human monocytes treated with IL-13.²⁵ Interestingly, ENMs alone could modify macrophage phenotype. For example, alveolar macrophages from rats exposed to CeO₂ nanoparticles had increased levels of arginase-1 mRNA, which is a marker of AAMs.²⁶

Macrophage activation by ENMs involves a complex network of intracellular signaling pathways, some of which are designed as protective responses to oxidative stress and cell injury. Specific types of ENMs have been reported to have either enhanced or suppressed ROS-mediated events in macrophages. For example, Ag nanoparticles increase the expression of NF- κ B and COX-2 in RAW264.7 macrophages, whereas no such pro-inflammatory effect was found with Au nanoparticles.²⁷ Multi-walled CNTs also increase levels of the COX-2 enzyme through a MAP kinase-dependent signaling pathway in mouse RAW264.7 cells *in vitro*.²⁸ Additionally, the antioxidant mediator Nrf2 is activated by multi-walled CNTs in cultured human THP-1 cells.²⁹ Both COX-2 and Nrf2 are protective factors against lung disease that are increased to counteract ROS-induced cellular stress initiated by CNTs. In contrast to multi-walled CNTs, platinum (Pt) nanoparticles suppress inflammatory responses of RAW264.7 cells by reducing bacterial lipopolysaccharide (LPS) induction of ROS, ERK phosphorylation and levels of COX-2 and iNOS.³⁰ These studies collectively suggest that the effects of ENMs, on either inducing or suppressing pro-inflammatory signaling pathways in macrophages, could be determined by multiple factors including shape, elemental composition, and ROS-generating potential of the ENM.

Nanoparticle effects on pre-existing airway disease

ENMs will most likely have the most profound adverse health effects on individuals with pre-existing respiratory diseases such as asthma, bronchitis, or COPD.^{31,32} For example,

multi-walled CNTs exacerbate allergic airway inflammation in mice caused by ovalbumin sensitization as determined by amplified lung levels of Th2 cytokines and chemokines, as well as serum IgE levels, compared with allergen alone.³³ Single-walled CNTs have also been reported to exacerbate allergic airway inflammation in mice via enhanced activation of Th immunity and increased oxidative stress.^{34,35} In addition to airway inflammation, CNTs have also been shown to exacerbate airway fibrosis in mice that were first pre-challenged with ovalbumin. However, in this study lung levels of allergen-induced IL-13, a principal Th2 cytokine, were reduced by multi-walled CNT exposure compared to allergen alone.³⁶ Repeated exposure to multi-walled CNTs has also been shown to induce Th2 allergic responses in the absence of any allergen pre-exposure.³⁷ Other ENMs, besides multi-walled CNTs, have also been shown to exacerbate allergic inflammation in mice. TiO₂ or Au nanoparticles enhance airway hyper-responsiveness in a mouse model of diisocyanate-induced airway inflammation and increase numbers of inflammatory cells.³⁸ However, the majority of inflammatory cells in that study were neutrophils, which suggests a shift from the classic Th2 response to one that primarily features Th17-mediated inflammation. While these studies suggest that individuals with allergic asthma are susceptible to lung and airway disease caused by ENMs exposure, it remains unknown whether ENMs will cause or exacerbate asthma in humans. Some of the effects of ENMs (metal nanoparticles or nanotubes) on the modulation of allergen-induced airway remodeling are illustrated in Fig. 1.

While there is a significant body of evidence in rodents suggesting that ENMs (e.g., CNTs) would be a hazard to individuals with asthma through exacerbation of Th2 inflammation, some studies with ENMs show suppression of allergen-induced airway disease in mice. For example, it has been shown that TiO₂ nanoparticles cause neutrophilic inflammation in healthy mice, but suppresses allergic airway inflammation in mice sensitized with ovalbumin allergen.³⁹ Therefore, there is evidence that ENMs can cause either immunostimulation or immunosuppression.

Dendritic cells (DCs) are important immune initiators that serve to capture and present allergens to naïve T cells, thereby driving T cell polarization.⁴⁰ Therefore, understanding the effects of ENMs on the function and phenotype of DCs is an important area of study. The exacerbation of allergen-induced airway disease discussed above could be partly through the inappropriate activation of antigen-presenting DCs.³³ Both ENMs and diesel pollutant nanoparticles have been shown to activate DCs where as other work has shown that ZnO nanoparticles cause DC death at relatively low concentrations.^{41,42} Interestingly though, CNTs have been reported to inhibit the differentiation of peripheral blood monocytes into DCs.⁴³ Mast cells are also important in the allergic immune response.⁴⁴ Recently, mast cells have been shown to participate in the activation of the IL-33/ST2 axis to mediate adverse pulmonary and cardiovascular responses to multi-walled CNTs.⁴⁵

The effects of ENMs in the lung could also be exacerbated by pre-existing bacterial or viral infection. Bacterial LPS is a potent pro-inflammatory agent and has been implicated in a number of occupational and environmental lung diseases in humans, including bronchitis, chronic obstructive pulmonary disease (COPD), and asthma. CNTs, either single- or multi-walled, have been reported to increase the severity of LPS-induced lung inflammation,

pulmonary vascular permeability, and production of pro-inflammatory cytokines in the lungs of mice.⁴⁶ Moreover, LPS pre-exposure increased pulmonary fibrosis induced by multi-walled CNTs as well as CNT-induced production of PDGF by rat alveolar macrophages and lung epithelial cells.⁴⁷ These studies provide evidence that LPS-induced lung inflammation is a susceptibility factor that increases the severity of fibroproliferative lung disease caused by CNT exposure.

Pulmonary fibrogenesis induced by nanoparticles

Pulmonary fibrosis is an occupational hazard following exposure to many particles and fibers. Innate immune responses could play an important role in the progression or resolution of fibrosis. ENMs, because of their increased surface area to mass ratio and ROS-producing potential, could pose a significant risk for the development of fibrosis. Certain assumptions have been made with respect to ENM toxicity and expected disease outcome. For example, CNTs share some features with asbestos fibers, mainly with regard to their fiber-like shape and aspect (length to width) ratio. Asbestos fibers are a known cause of fibrosis and mesothelioma in humans. However, CNTs have some uniquely different properties from asbestos, including nanoscale width and highly conformal structure. Therefore, some caution should be taken in making comparisons of fiber-like or tube-shaped ENMs with asbestos fibers with respect to fibrogenic potential. Nevertheless, pulmonary fibrosis is a common pathologic feature observed in numerous rodent studies after exposure to CNTs.⁴⁸⁻⁵⁰ As mentioned above, aggregated CNTs tend to produce granulomatous lesions.^{49,51-53} In contrast, diffuse interstitial pulmonary fibrosis is associated with well-dispersed CNTs.⁵⁴ Moreover, well-dispersed multi-walled CNTs were readily taken up by macrophages and cause greater growth factor (PDGF-AA, TGF- β 1) and IL-1 β production than non-dispersed CNTs.⁵⁴ Other factors contribute to the fibrogenic potential of ENMs, including shape, composition, electrostatic charge, and ROS generating capacity. High aspect ratio ENMs impede clearance and structures longer than ten to fifteen micrometers (the approximate width of an alveolar macrophage) are difficult to clear from lung tissues via macrophage-mediated mechanisms. For fiber or tube-shaped ENMs, diameter and rigidity are also determinants of toxicity. For example, thinner (~10 nm diameter) multi-walled CNTs are more toxic in the lungs of mice than thicker (~70 nm diameter) multi-walled CNTs.⁵⁵ However, long single-walled CNT are flexible when folded and are taken up by macrophages without causing frustrated phagocytosis or impeding macrophage clearance. Furthermore, the composition of ENMs must also be carefully considered. For example, metals used as catalysts in the manufacture of CNTs (e.g., nickel, cobalt, iron) are known to mediate pulmonary fibrosis in humans.⁵⁶ Studies have determined that nickel can cause occupational asthma and contact dermatitis, whereas iron and cobalt cause interstitial pulmonary fibrosis in occupations related to mining and metallurgy.

The emergence of the myofibroblast, a collagen synthetic mesenchymal cell, is a key step in the progression of lung fibrosis. A variety of growth factors, cytokines, and chemokines that stimulate myofibroblast differentiation, growth, migration, and extracellular matrix production are induced in the lungs of rats or mice after exposure to ENMs.⁵⁷ Some of these mediators and their relationship to lung region and cell type are shown in Fig. 1. For example, single- or multi-walled CNTs delivered to the lung by intratracheal instillation in

rats or inhalation in mice increase mRNA and protein levels of PDGF.^{13,36,47} PDGF stimulates the replication, chemotaxis, and survival of lung mesenchymal cells (fibroblasts, myofibroblasts, and smooth muscle cells) to promote lung fibrogenesis.⁵⁸ CNTs delivered to the lungs of mice increase levels of TGF- β 1, a central mediator of collagen production by fibroblasts and myofibroblasts.^{49,54} In addition to TGF- β 1, osteopontin (OPN) levels stimulate collagen deposition and fibroblast migration and are increased in the lungs of rats exposed to single-walled CNTs.¹³ Alveolar macrophages, as well as airway epithelial cells and fibroblasts, produce PDGF, TGF- β 1, and OPN. Additionally, several chemokines are also induced by CNT exposure and drive the inflammatory response in the lung. CXCL8 (IL-8), a potent neutrophil chemoattractant, is produced by a human bronchial epithelial cell line *in vitro* after exposure to multi-walled CNTs.⁵⁹ CCL2, also known as monocyte chemoattractant protein-1 (MCP-1), is produced by macrophages and airway epithelial cells and is increased in the bronchoalveolar lavage fluid of mice after CNT inhalation exposure.³⁶ In general, a complex interaction of cytokines, chemokines and growth factors contributes to the progression of pulmonary fibrosis.

Pleural immune responses to nanoparticles

In addition to airway and interstitial lung diseases, the pleural mesothelial lining surrounding the lungs is a potentially important site of toxicity for certain ENMs. Of particular concern are high aspect ratio ENMs such as CNTs, nanofibers, and nanowires that have asbestos-like shape and therefore could be persistent in lung tissue. ENMs contained within macrophage cross the pleural lining via the lymphatic drainage and thereby interact with the mesothelial lining of the pleura. Here, the durable nature of CNTs, nanofibers, or nanowires, coupled with fiber-like shape and reactivity (i.e., ROS-generating capacity), could result in immune reactions, pleural inflammation, or DNA damage to mesothelial cells. While unknown at the present time, it has been speculated that such high-aspect ratio ENMs could have asbestos-like behavior and long term immune or inflammatory effects that could lead to tumor formation (i.e., mesothelioma). Researchers have demonstrated that intraperitoneal injection of long multi-walled CNTs in mice induces inflammation and granuloma formation on the mesothelial surface of the peritoneum.⁶⁰ A similar strategy with CNTs, using mice deficient in the tumor suppressor p53, showed mesothelioma formation in the abdominal cavity after injection of CNTs.⁶¹ CNTs also activate p53 in mouse embryonic stem cells *in vitro*.⁶² Furthermore, multi-walled CNTs delivered to the lungs by inhalation or aspiration accumulate in subpleural tissue with some tubes penetrating the pleural lining.^{9,50,63} The inhalation of multi-walled CNTs in mice has been shown to produce pro-inflammatory lesions on the pleural surface, that have been referred to as mononuclear cell aggregates.⁹ Additionally, these same mice had elevated levels of PDGF and CCL2 in their bronchoalveolar lavage fluid. Interestingly, PDGF and Ni nanoparticles synergistically increase CCL2 production by cultured rat mesothelial cells.⁶⁴ The mechanism involves Ni enhancement of PDGF-induced CCL2 expression by prolonged MAP kinase activation in mesothelial cells. Illustrated in Fig. 2 is a scheme that depicts the formation of pleural mononuclear cell aggregates following exposure to CNTs or Ni nanoparticles in mice *in vivo*, along with a hypothetical model of ENM-induced CCL2 expression by mesothelial cells. The accumulation of mononuclear cell aggregates at the pleural surface after exposure

to CNTs, or presumably Ni nanoparticles, could be mediated by PDGF secreted from activated macrophages. PDGF, in turn, stimulates the production of CCL2 that serves to recruit mononuclear cells to the pleural surface. Moreover, CCL2 is produced by pleural mesothelial cells and is a candidate chemokine that could participate in the formation of mononuclear cell aggregates observed at the pleura of mice after inhalation of multi-walled CNTs.^{9,65} The issue of whether ENMs are capable of causing immune, inflammatory, or carcinogenic effects at the pleura in humans remains a key topic of research and will have important implications for the future use and development of ENMs for a variety of applications.

Effects of nanoparticles moving across the lung epithelial barrier

While macrophages represent the first line of immune defense against inhaled ENMs, a variety of other pulmonary cell types contribute to innate immunity. For example, the lung epithelium comprises an enormous surface area for ENM exposure and *in vivo* and *in vitro* studies show that epithelial cells are a primary target for ENM-induced oxidative stress.^{66,67} Innovative aerosolization systems have been developed to expose lung epithelial cells *in vitro* to ENMs in air-liquid interface, rather than submerged cell cultures, to accurately model nanoparticle toxicity in the lung.⁶⁸ Airway epithelial cells play an important defense role by producing mucus which provides a protective barrier against inhaled microbes. Most ENMs, including CNTs and metal oxide nanoparticles, are trapped in the mucus layer.⁶⁹ However, recent findings suggest that certain muco-adhesive ENMs can disrupt mucus barriers and cause greater exposure to foreign particles, including pathogens and other potentially toxic nanomaterials.⁷⁰ While disruption of the mucus barrier could be detrimental, the ability of certain ENMs to penetrate mucus could be exploited for drug delivery in a controlled manner for the treatment of airway diseases such as cystic fibrosis and asthma. The ability of ENMs to penetrate mucus and the epithelial layers likely depends on a number of factors, including nanoparticle size, surface charge and hydrophobicity. In contrast to the airway epithelial barrier, the alveolar epithelial barrier is relatively thin. As such, ENMs have the potential to cross the epithelial-endothelial barrier, access the blood capillaries and then distribute to other organ systems.⁷¹

Systemic immune responses to inhaled nanoparticles

ENMs are capable of activating immune responses in tissues and organ systems beyond the lung, including the spleen and heart. For example, inhaled multi-walled CNTs cause systemic immunosuppression in mice through a mechanism that involves the release of TGF- β 1 from the lungs, which enters the bloodstream to signal COX-2-mediated increases in PGE₂ and IL-10 in the spleen, both of whose function is to suppress T cell proliferation.^{72,73} Also, single-walled CNTs or multi-walled CNTs delivered to the lungs of mice have been reported to exacerbate cardiovascular dysfunction and disease.⁷⁴ However, the systemic effects of these CNTs were likely due to the release of soluble cytokines or growth factors from the lungs as there was no evidence of CNT translocation from the lung. Nevertheless, it is possible that at least some ENMs will translocate from the lung to distant organs to modulate immune responses.

Nanoparticles for the treatment of lung disease

Presently, there is great interest in utilizing nanotechnology to both develop new pharmaceuticals as well as to address issues with drug delivery. The unique properties of ENMs make them ideal candidates for the development of novel treatment strategies for a variety of lung diseases. Their nanoscale size and high degree of functionalization fosters interactions with biomolecules within the cell as well as those on the cell surface, making drug delivery to cells within the lungs highly specific, offering local targeting for the treatment of lung diseases such as asthma, cancer, and bacterial infection.⁷⁵ These drug delivery platforms include but are not limited to carbon based (fullerenes, dendrimers)⁷⁶ and silica based platforms used to treat lung cancer,⁷⁷ as well as metal-based platforms used to treat mucus hypersecretion in allergic airway inflammation.⁷⁸ While each of these platforms offer a novel approach to the treatment of some very problematic illnesses, careful consideration should be given to the potential risks of these systems in order to ensure that therapeutic benefits outweigh the possibility of immunotoxicity.

Conclusions and summary

The emergence of nanotechnology has generated a wide spectrum of novel ENMs that will revolutionize engineering, electronics, and medicine. However, it is essential to address the potential human health risks that ENMs pose in occupational, consumer, or environmental exposure scenarios since their chemical and biological reactivities are unpredictable. Immune reactions are a key concern for potential adverse effects of ENMs along with concerns that certain ENMs will have the potential to cause fibrosis and/or cancer in humans based on physical similarities to toxic fibers and growing evidence that CNTs cause fibrosis and perhaps cancer in rodents. While certain predictions can be made regarding the toxicity of ENMs, the unique nature of these structures make it difficult, if not impossible, to predict how some ENMs will interact with intracellular structures such as DNA, cell membranes, and cytoskeletal proteins. ENM exposure has not yet been linked to human disease due to the relatively recent emergence of nanotechnology and the lack of exposure or epidemiologic data. However, it is likely that the most susceptible to adverse immune effects of ENMs will be individuals with preexisting respiratory disease such as asthma or chronic bronchitis.

Acknowledgments

This work was supported by U.S. Public Health Service Grants R01ES020897 and RC2-ES018772.

References

1. Card JW, Zeldin DC, Bonner JC, et al. Pulmonary applications and toxicity of engineered nanoparticles. *Am J Physiol*. 2008; 295(3):L400–11.
2. Bonner JC. Nanoparticles as a potential cause of pleural and interstitial lung disease. *Proc Am Thorac Soc*. 2010; 7(2):138–41. [PubMed: 20427587]
3. Hussain S, Vanoirbeek JA, Hoet PH. Interactions of nanomaterials with the immune system. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2012; 4(2):169–83. [PubMed: 22144008]
4. Dobrovolskaia MA, McNeil SE. Immunological properties of engineered nanomaterials. *Nat Nanotechnol*. 2007; 2(8):469–78. [PubMed: 18654343]

5. Ma-Hock L, Treumann S, Strauss V, et al. Inhalation toxicity of multiwall carbon nanotubes in rats exposed for 3 months. *Toxicol Sci.* 2009; 112(2):468–81. [PubMed: 19584127]
6. Pauluhn J. Subchronic 13-week inhalation exposure of rats to multiwalled carbon nanotubes: toxic effects are determined by density of agglomerate structures, not fibrillar structures. *Toxicol Sci.* 2010; 113(1):226–42. [PubMed: 19822600]
7. Shvedova AA, Kisin E, Murray AR, et al. Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis. *Am J Physiol.* 2008; 295(4):L552–65.
8. Mercer RR, Scabilloni J, Wang L, et al. Alteration of deposition pattern and pulmonary response as a result of improved dispersion of aspirated single-walled carbon nanotubes in a mouse model. *Am J Physiol.* 2008; 294:L87–97.
9. Ryman-Rasmussen JP, Cesta MF, Brody AR, et al. Inhaled multi-walled carbon nanotubes reach the subpleural tissue in mice. *Nat Nanotechnol.* 2009; 4(11):747–51. [PubMed: 19893520]
10. Bonner, JC. *Respiratory Toxicology in Molecular and Biochemical Toxicology*. 4th. Smart, RC.; Hodgson, E., editors. Vol. 2008. John Wiley & Sons, Inc; New York: p. 639-70.
11. Donaldson K, Murphy FA, Duffin R, et al. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. *Part Fibre Toxicol.* 2010; 7:5. [PubMed: 20307263]
12. Murphy FA, Schinwald A, Poland CA, et al. The mechanism of pleural inflammation by long carbon nanotubes: interaction of long fibres with macrophages stimulates them to amplify pro-inflammatory responses in mesothelial cells. *Part Fibre Toxicol.* 2012; 9:8. [PubMed: 22472194]
13. Mangum JB, Turpin EA, Antao-Menezes A, et al. Single-walled carbon nanotube (SWCNT)-induced interstitial fibrosis in the lungs of rats is associated with increased levels of PDGF mRNA and the formation of unique intercellular carbon structures that bridge alveolar macrophages in situ. *Part Fibre Toxicol.* 2006; 3:15. [PubMed: 17134509]
14. Liu R, Zhang X, Pu Y, et al. Small-sized titanium dioxide nanoparticles mediate immune toxicity in rat pulmonary alveolar macrophages in vivo. *J Nanoscale Nanotechnol.* 2010; 10(8):5161–9.
15. Shvedova AA, Fabisiak JP, Kisin ER, et al. Sequential exposure to carbon nanotubes and bacteria enhances pulmonary inflammation and infectivity. *Am J Respir Cell Mol Biol.* 2008; 38(5):579–90. [PubMed: 18096873]
16. Hussain S, Al-Nsour F, Rice AB, et al. Cerium dioxide nanoparticles induce apoptosis and autophagy in human peripheral blood monocytes. *ACS Nano.* 2012; 6(7):5820–9. [PubMed: 22717232]
17. Palomäki J, Välimäki E, Sund J, et al. Long, needle-like carbon nanotubes and asbestos activate the NLRP3 inflammasome through a similar mechanism. *ACS Nano.* 2011; 5(9):6861–70. [PubMed: 21800904]
18. Meunier E, Coste A, Olganier D, et al. Double-walled carbon nanotubes trigger IL-1 β release in human monocytes through Nlrp3 inflammasome activation. *Nanomedicine.* 2012; 8(6):987–95. [PubMed: 22100755]
19. Sun B, Wang X, Ji Z, et al. nlrp3 inflammasome activation induced by engineered nanomaterials. *Small.* 2013; 9(9-10):1595–607. [PubMed: 23180683]
20. Strowig T, Henao-Mejia J, Elinav E, et al. Inflammasomes in health and disease. *Nature.* 2012; 481(7381):278–86. [PubMed: 22258606]
21. Hamilton RF Jr, Buford M, Xiang C, et al. NLRP3 inflammasome activation in murine alveolar macrophages and related lung pathology is associated with MWCNT nickel contamination. *Inhal Toxicol.* 2012; 24(14):995–1008. [PubMed: 23216160]
22. Wang YZ, Zhang P, Rice AB, et al. Regulation of interleukin-1 β -induced platelet-derived growth factor receptor- α expression in rat pulmonary myofibroblasts by p38 mitogen-activated protein kinase. *J Biol Chem.* 2000; 275(29):22550–7. [PubMed: 10807932]
23. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. *Annu Rev Immunol.* 2009; 27:229–65. [PubMed: 19302040]
24. Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity.* 2010; 32(5):593–604. [PubMed: 20510870]

25. Scotton CJ, Martinez FO, Smelt MJ, et al. Transcriptional profiling reveals complex regulation of the monocyte IL-1 beta system by IL-13. *J Immunol.* 2005; 174(2):834–45. [PubMed: 15634905]
26. Ma JY, Zhao H, Mercer RR, et al. Cerium oxide nanoparticle-induced pulmonary inflammation and alveolar macrophage functional change in rats. *Nanotoxicology.* 2011; 5(3):312–25. [PubMed: 20925443]
27. Nishanth RP, Jyotsna RG, Schlager JJ, et al. Inflammatory responses of RAW 264.7 macrophages upon exposure to nanoparticles: role of ROS-NFκB signaling pathway. *Nanotoxicology.* 2011; 5(4):502–16. [PubMed: 21417802]
28. Lee JK, Sayers BC, Chun KS, et al. Multi-walled carbon nanotubes induce COX-2 and iNOS expression via MAP kinase-dependent and -independent mechanisms in mouse RAW264.7 macrophages. *Part Fibre Toxicol.* 2012; 9:14. [PubMed: 22571318]
29. Brown DM, Donaldson K, Stone V. Nuclear translocation of Nrf2 and expression of antioxidant defence genes in THP-1 cells exposed to carbon nanotubes. *J Biomed Nanotechnol.* 2010; 6(3): 224–33. [PubMed: 21179939]
30. Rehman MU, Yoshihisa Y, Miyamoto Y, et al. The anti-inflammatory effects of platinum nanoparticles on the lipopolysaccharide-induced inflammatory response in RAW264.7 macrophages. *Inflamm Res.* 2012; 61(11):1177–85. [PubMed: 22752115]
31. Bonner JC. Carbon nanotubes as delivery systems for respiratory disease: do the dangers outweigh the potential benefits? *Expert Rev Respir Med.* 2011; 5(6):779–87. [PubMed: 22082164]
32. Inoue K, Takano H. (2011) Aggravating impact of nanoparticles on immune-mediated pulmonary inflammation. *ScientificWorldJournal.* 2011; 11:382–90. [PubMed: 21336454]
33. Inoue K, Koike E, Yanagisawa R, et al. Effects of multi-walled carbon nanotubes on a murine allergic airway inflammation model. *Toxicol Appl Pharmacol.* 2009; 237(3):306–16. [PubMed: 19371758]
34. Inoue K, Yanagisawa R, Koike E, et al. Repeated pulmonary exposure to single-walled carbon nanotubes exacerbates allergic inflammation of the airway: Possible role of oxidative stress. *Free Radic Biol Med.* 2010; 48(7):924–34. [PubMed: 20093178]
35. Nygaard UC, Hansen JS, Samuelsen M, et al. Single-walled and multi-walled carbon nanotubes promote allergic immune responses in mice. *Toxicol Sci.* 2009; 109(1):113–23. [PubMed: 19293371]
36. Ryman-Rasmussen JP, Tewksbury EW, Moss OR, et al. Inhaled multiwalled carbon nanotubes potentiate airway fibrosis in a murine model of allergic asthma. *Am J Resp Cell Mol Biol.* 2009; 40(3):349–58.
37. Park EJ, Cho WS, Jeong J, et al. Pro-inflammatory and potential allergic responses resulting from B cell activation in mice treated with multi-walled carbon nanotubes by intratracheal instillation. *Toxicology.* 2009; 259(3):113–21. [PubMed: 19428951]
38. Hussain S, Vanoirbeek JA, Luyts K, et al. Lung exposure to nanoparticles modulates an asthmatic response in a mouse model. *Eur Respir J.* 2011; 37(2):299–309. [PubMed: 20530043]
39. Rossi EM, Pytkäinen L, Koivisto AJ, et al. Inhalation exposure to nanosized and fine TiO₂ particles inhibits features of allergic asthma in a murine model. *Part Fibre Toxicol.* 2010; 7:35. [PubMed: 21108815]
40. Gill MA. The role of dendritic cells in asthma. *J Allergy Clin Immunol.* 2012; 129(4):889–901. [PubMed: 22464668]
41. Bezemer GF, Bauer SM, Oberdörster G, et al. Activation of pulmonary dendritic cells and Th2-type inflammatory responses on instillation of engineered, environmental diesel emission source or ambient air pollutant particles in vivo. *J Innate Immun.* 2011; 3(2):150–66. [PubMed: 21099199]
42. Andersson-Willman B, Gehrmann U, Cansu Z, et al. Effects of subtoxic concentrations of TiO₂ and ZnO nanoparticles on human lymphocytes, dendritic cells and exosome production. *Toxicol Appl Pharmacol.* 2012; 264(1):94–103. [PubMed: 22842014]
43. Laverny G, Casset A, Purohit A, et al. Immunomodulatory properties of multi-walled carbon nanotubes in peripheral blood mononuclear cells from healthy subjects and allergic patients. *Toxicol Lett.* 2012; 217(2):91–101. [PubMed: 23266719]
44. Brown JM, Wilson TM, Metcalfe DD. The mast cell and allergic diseases: role in pathogenesis and implications for therapy. *Clin Exp Allergy.* 2008; 38(1):4–18. [PubMed: 18031566]

45. Katwa P, Wang X, Urankar RN, et al. A carbon nanotube toxicity paradigm driven by mast cells and the IL33/ST₂ axis. *Small*. 2012; 8(18):2904–12. [PubMed: 22777948]
46. Inoue K, Takano H, Koike E, et al. Effects of pulmonary exposure to carbon nanotubes on lung and systemic inflammation with coagulatory disturbance induced by lipopolysaccharide in mice. *Exp Biol Med*. 2008; 233(12):1583–90.
47. Cesta MF, Ryman-Rasmussen JP, Wallace DG, et al. Bacterial lipopolysaccharide enhances PDGF signaling and pulmonary fibrosis in rats exposed to carbon nanotubes. *Am J Respir Cell Mol Biol*. 2010; 43(2):142–51. [PubMed: 19738159]
48. Li JG, Li WX, Xu JY, et al. Comparative study of pathological lesions induced by multiwalled carbon nanotubes in lungs of mice by intratracheal instillation and inhalation. *Environ Toxicol*. 2007; 22(4):415–21. [PubMed: 17607736]
49. Shvedova AA, Kisin ER, Mercer R, et al. Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. *Am J Physiol Lung Cell Mol Physiol*. 2005; 289(5):L698–708. [PubMed: 15951334]
50. Porter DW, Hubbs AF, Mercer RR, et al. Mouse pulmonary dose- and time course-responses induced by exposure to multi-walled carbon nanotubes. *Toxicology*. 2010; 269(2-3):136–47. [PubMed: 19857541]
51. Muller J, Huaux F, Moreau N, et al. Respiratory toxicity of multi-wall carbon nanotubes. *Toxicol Appl Pharmacol*. 2005; 207(3):221–31. [PubMed: 16129115]
52. Murray AR, Kisin ER, Tkach AV, et al. Factoring-in agglomeration of carbon nanotubes and nanofibers for better prediction of their toxicity versus asbestos. *Part Fibre Toxicol*. 2012; 9:10. [PubMed: 22490147]
53. Warheit DB, Laurence BR, Reed KL, et al. Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicol Sci*. 2004; 77(1):117–25. [PubMed: 14514968]
54. Wang X, Tian X, Ntim SA, et al. The dispersal state of multi-walled carbon nanotubes influences pro-fibrogenic epithelial and macrophage responses that correlate with the extent of pulmonary fibrosis. *ACS Nano*. 2011; 5(12):9772–87. [PubMed: 22047207]
55. Fenoglio I, Aldieri E, Gazzano E, et al. Thickness of multiwalled carbon nanotubes affects their lung toxicity. *Chem Res Toxicol*. 2012; 25(1):74–82. [PubMed: 22128750]
56. Kelleher P, Pacheco K, Newman LS. Inorganic dust pneumonias: the metal-related parenchymal disorders. *Environ Health Perspect*. 2000; 108(suppl 4):685–96. [PubMed: 10931787]
57. Bonner JC. Mesenchymal cell survival in airway and interstitial pulmonary fibrosis. *Fibrogenesis Tissue Repair*. 2010; 3:15. [PubMed: 20738867]
58. Bonner JC. Regulation of PDGF and its receptors in fibrotic diseases. *Cytokine Growth Factor Rev*. 2004; 15:255–73. [PubMed: 15207816]
59. Hirano S, Fujitani Y, Furuyama A, et al. Uptake and cytotoxic effects of multi-walled carbon nanotubes in human bronchial epithelial cells. *Toxicol Appl Pharmacol*. 2010; 249(1):8–15. [PubMed: 20800606]
60. Poland CA, Duffin R, Kinloch I, et al. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nat Nanotechnol*. 2008; 3(7):423–28. [PubMed: 18654567]
61. Takagi A, Hirose A, Nishimura T, et al. Induction of mesothelioma in p53 +/- mouse by intraperitoneal application of multi-wall carbon nanotube. *J Toxicol Sci*. 2008; 33(1):105–16. [PubMed: 18303189]
62. Zhu L, Chang DW, Dai L, et al. DNA damage induced by multiwalled carbon nanotubes in mouse embryonic stem cells. *Nano Lett*. 2007; 7(12):3592–7. [PubMed: 18044946]
63. Mercer RR, Hubbs AF, Scabilloni JF, et al. Distribution and persistence of pleural penetrations by multi-walled carbon nanotubes. *Part Fibre Toxicol*. 2010; 7:28. [PubMed: 20920331]
64. Glista-Baker EE, Taylor AJ, Sayers BC, et al. Nickel nanoparticles enhance platelet-derived growth factor-induced chemokine expression by mesothelial cells via prolonged mitogen-activated protein kinase activation. *Am J Respir Cell Mol Biol*. 2012; 47(4):552–61. [PubMed: 22700867]
65. Visser CE, Tekstra J, Brouwer-Steenbergen JJ, et al. (1998) Chemokines produced by mesothelial cells: huGRO-alpha, IP-10, MCP-1 and RANTES. *Clin Exp Immunol*. 1998; 112(2):270–5. [PubMed: 9649190]

66. Creutzenberg O. Biological interactions and toxicity of nanomaterials in the respiratory tract and various approaches of aerosol generation for toxicity testing. *Arch Toxicol.* 2012; 86(7):1117–22. [PubMed: 22418596]
67. Geiser M, Kreyling WG. Deposition and biokinetics of inhaled nanoparticles. *Part Fibre Toxicol.* 2010; 7:2. [PubMed: 20205860]
68. Paur HR, Cassee FR, Teeguarden J, et al. In-vitro cell exposure studies for the assessment of nanoparticle toxicity in the lung—A dialog between aerosol science and biology. *J Aerosol Science.* 2011; 42(10):668–92.
69. Jachak A, Lai SK, Hida K, et al. Transport of metal oxide nanoparticles and single-walled carbon nanotubes in human mucus. *Nanotoxicology.* 2012; 6(6):614–22. [PubMed: 21800953]
70. Wang YY, Lai SK, So C, et al. Mucoadhesive nanoparticles may disrupt the protective human mucus barrier by altering its microstructure. *PLoS One.* 2011; 6(6):e21547. [PubMed: 21738703]
71. Kreyling WG, Hirn S, Schleh C. Nanoparticles in the lung. *Nat Biotechnol.* 2010; 28(12):1275–6. [PubMed: 21139613]
72. Mitchell LA, Gao J, Wal RV, et al. Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes. *Toxicol Sci.* 2007; 100(1):203–14. [PubMed: 17660506]
73. Mitchell LA, Lauer FT, Burchiel SW, et al. Mechanisms for how inhaled multiwalled carbon nanotubes suppress systemic immune function in mice. *Nature Nanotech.* 2009; 4(7):451–6.
74. Erdely A, Hulderman T, Salmen R, et al. Cross-talk between lung and systemic circulation during carbon nanotube respiratory exposure. Potential biomarkers *Nano Lett.* 2009; 9(1):36–43.
75. Sung JC, Pulliam BL, Edwards DA. Nanoparticles for drug delivery to the lungs. *Trends Biotechnol.* 2007; 25(12):563–70. [PubMed: 17997181]
76. Bharali DJ, Khalil M, Simone TM, et al. Nanoparticles and Cancer therapy: A concise review with emphasis on dendrimers. *Int J Nanomedicine.* 2009; 4:1–7. [PubMed: 19421366]
77. Liu Q, Zhang J, Sun W, et al. Delivering hydrophilic and hydrophobic chemotherapeutics simultaneously by magnetic meoporous silica nanoparticles to inhibit cancer cells. *Int J Nanomedicine.* 2012; 7:999–1013. [PubMed: 22403484]
78. Jang S, Park JW, Cha HR, et al. Silver nanoparticles modify VEGF signaling pathway and mucus hypersecretion in allergic airway inflammation. *Int J Nanomedicine.* 2012; 7:1329–43. [PubMed: 22457593]

Figure 1.

Illustration summarizing some of the effects of metal oxide nanoparticles (NPs) or carbon nanotubes (CNTs) on airway remodeling in asthma. In normal healthy lungs, Metal NPs (e.g., TiO₂, NiO, ZnO) or CNTs (single- or multi-walled CNTs) stimulate a Th1 inflammatory response characterized by IFN- γ production, classic macrophage activation, neutrophilic infiltration, and fibrosis. Under conditions of pre-existing allergen-induced airway inflammation (i.e., asthma), NPs or CNTs enhance allergen-induced mucus production, modulate allergen-induced airway fibrosis, and alter allergen-induced Th2 responses and eosinophilic inflammation to produce a mixed inflammatory cell immune response.

Figure 2.

Illustration showing postulated effects of carbon nanotubes (CNTs) or nickel nanoparticles (NiNPs) on pleural inflammation. CNTs or NiNPs taken up by macrophages traffic to the subpleura or exit the lung via lymphatic ducts and produce PDGF to signal mesothelial cell secretion of chemokines (CCL2, CXCL10) that mediate mononuclear cell accumulation at the pleural surface (see ref 9). The inset shows the interaction between a CNT- or NiNP-activated macrophages and a mesothelial cell. Macrophage-derived PDGF stimulates MAPK activation in mesothelial cells and NiNPs prolong PDGF-induced MAP kinase activation, leading to synergistic increases in chemokine production (see ref 64).