Molecular and Integrative Toxicology

James C. Bonner
Jared M. Brown *Editors*

Interaction of Nanomaterials with the Immune System

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Preface

Nanotechnology is manipulation of matter at the atomic scale for a plethora of applications, some of which hold solutions for our most pressing challenges, such as energy and medicine.

Despite the enormous potential benefits, there is also the potential danger that the advancement of the nanotechnology industry will bring with it adverse human health effects. One recognized effect is the impact of engineered nanomaterials (ENMs) on the immune system as these materials are foreign to the human body. The focus of this book is to present an overview of the principles and basic mechanisms of immunotoxicity caused by ENMs. Human exposure to ENMs occurs occupationally at workplaces, as a result of specific biomedical or consumer applications, or after environmental contamination resulting from nanomaterials released into the air, water, and soil.

The impact of ENMs on the human immune system has yet to be determined. This is due to the relatively recent emergence of the nanotechnology industry over the past few decades. However, the evidence from global studies using rodents or cultured human cells, some of which is presented in this book, predicts that ENMs will cause some degree of immune-related diseases in humans, including but perhaps not limited to allergies, asthma, hypersensitivity reactions, autoimmune disease, fibrosis, and cancer. For this reason, we feel that this book is timely and deals with key issues for understanding ENM interaction with the immune system that will help us proactively prevent future immune-related diseases.

ENMs, like other specific types of chemicals, influence the immune system upon inhalation, ingestion, injection, and dermal exposure. However, unlike many chemicals, ENMs deserve some special attention due to their unique interactions with biological systems. For example, the term *nano-bio interface* was coined to encompass the interaction of ENMs with biomolecules, cell membranes, or intracellular components (e.g., actin, DNA). These interactions at the subcellular scale make ENMs unique, for better or worse, and emphasize the concept that size does indeed matter. It is not our intent to present information on nanomedicine applications, although some overlap with this topic is inevitable due to immunotoxic side effects of some nanotherapeutics.

Some valuable documents worth mentioning are already available on immunotoxicity caused by chemical exposure and on methods for assessing immunotoxicity. For example, the topic of immunotoxicity and chronic disease caused by chemical exposure is a topic that has been addressed in a previous volume of *Molecular and Integrative Toxicology* (Dietert and Luebke 2012). Additionally, previous Environmental Health Criteria (EHC) documents published by the World Health Organization (WHO) have addressed chemical exposure and immunotoxicity. EHC monograph 212 of the International Programme on Chemical Safety (IPCS) focused on mechanisms, clinical aspects, epidemiology, hazard identification, and risk assessment of allergy and hypersensitivity following exposure to certain chemicals (IPCS 1999), while EHC monograph 236 focused on the induction of autoimmunity associated with chemical exposure (IPCS 2006). Finally, a forthcoming EHC monograph in 2020 entitled "Principles and Methods to Assess the Risk of Immunotoxicity Associated with Exposure to Nanomaterials" will present detailed information on testing methods (ICPS in press). Therefore, it is not our intent herein to provide a duplicative effort on immunotoxicity principles and testing methods but instead to illustrate mechanistic concepts of nanoimmunotoxicology from a diverse group of experts.

Finally, we are grateful to our scientific colleagues and friends who contributed to this book. The project was inspired by a shared interest and enthusiasm with our contributing colleagues that stemmed from formal scientific sessions, as well as informal conversations, at conferences in Europe and the USA. The chapters are authored by experts in the field of nanotechnology, toxicology, and immunology from six countries (Finland, the Netherlands, Sweden, Switzerland, the UK, and the USA). It is our hope that this book will provide some thought and guidance to the next generation of immunotoxicologists who will continue to address important issues related to nanotoxicology, human health, and the environment.

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Chapter 1
Introduction

James C. Bonner and Jared M. Brown

Abstract The rise of nanotechnology, a new industrial revolution, is generating a wealth of novel advanced materials that are dramatically changing the fields of electronics, engineering, and medicine. It is anticipated that these changes will solve important problems in renewable energy, more efficient communication and transportation systems, bioremediation of environmental pollution, and treatment of debilitating diseases. However, the impact of nanomaterials on the immune system is a concern, since manipulation of matter with a size range on par with subcellular structures has the potential to activate or suppress cells of the innate or adaptive immune system. This chapter overviews the topics covered in this book and thereby sets the stage for understanding the complexity of immune responses to a diversity of emerging engineered nanomaterials.

Keywords Nanotechnology · Immune system · Immunotoxicity

1.1 The Rapidly Expanding World of Engineered Nanomaterials (ENMs)

Nanotechnology, by a colloquial definition, is a new field of science focused on precision engineering of objects smaller than can be seen with the human eye. In more technical terms, nanotechnology may be defined as the design and manipulation of matter at the atomic scale to develop novel advanced materials. The potential benefits of nanotechnology are numerous, and include superior advances in electronics,

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highly efficient renewable energy cells that will replace and reduce conventional fossil fuel energy, nanoparticle-mediated remediation of environmental pollution, and new nano-enabled therapeutic approaches to treat deadly diseases such as cancer (Cattaneo et al. 2010). In order to maximize the success and benefits of nanotechnology, the design and synthesis of advanced nanomaterials should be considered with awareness of potential adverse effects on human health and the environment (Xia et al. 2009).

Like any new emerging technology, benefits are accompanied by risk. Some materials that are relatively innocuous at the macro- or larger scale might behave differently at the nanoscale. An example is titanium dioxide (TiO_2) that is relatively inert as a micron-sized particle, but as a nanomaterial can cause significant oxidative stress, inflammation, and tumor formation when instilled into the lungs of rodents (Oberdorster 1996). Also, some idea of relative toxicity or mode of action might be expected based on elemental composition alone, but some effects might be entirely unanticipated. For example, in elemental terms single-walled carbon nanotubes (SWCNTs) are essentially the same as graphite, a relatively nontoxic substance, but the dimensions of the nanotube structure (~1 to 4 nm width with a length >1 micrometer) are similar in scale to subcellular structures such as the cytoskeletal protein actin or a DNA molecule (Pampaloni and Florin 2008; Sargent et al. 2009). As such, there is the potential for nanotubes to interact with biomolecules with similar dimensions; e.g., wrapping or intertwining. This type of hybrid ENM–biomolecule interaction might explain some observed phenomena in immune cells; e.g., carbon bridge-like structures that form between alveolar macrophages in rats exposed to SWCNTs that do not occur upon exposure to spherical carbon nanoparticles (Mangum et al. 2006). ENMs also interact with biomolecules in the extracellular microenvironment to form a "*biocorona*" that influences the immune response (Neagu et al. 2017; Chen and Riviere 2017). In general, understanding the biophysicochemical interactions at the "*nano-bio interface"* is critical toward elucidating the impact of ENMs on cells of the immune system and whether these interactions could have biocompatible or bio-adverse outcomes on host immunity (Nel et al. 2009).

A major challenge for toxicologists and regulatory agencies in assessing risks associated with nanotechnology is the ever-increasing number and variety of ENMs. An important distinction to make at the forefront of this book is the difference between ENMs and anthropogenic nanoparticles (e.g., ambient ultrafine particles generated unintentionally as a by-product of man's activity). However, it has been acknowledged that much of what we know about ambient ultrafine particles can be applied to ENMs (Stone et al. 2017). The term *engineered nanomaterial* (ENM) may be more applicable than *engineered nanoparticle* (ENP) which refers to all dimensions and shapes <100 nm (e.g., spherical polyhedron and cube). Therefore, the abbreviation ENM is used throughout this book and includes ENPs as well as other structures with at least one dimension <100 nm (e.g., tubes and sheets).

1.2 Historical Perspective of Nanoparticle-Induced Immunotoxicity

It is not surprising that ENMs would present a challenge to our immune system. The mammalian immune system evolved to cope with foreign particles, many of them possessing nanoscale dimensions (between 1 and 100 nm), which presented a detrimental impact on the cellular and physiological well-being of the host. Foreign material entering the body, mainly through inhalation or ingestion, is identified as "not self" and dealt with accordingly. Some obvious invading "not-self" entities that accompanied the evolution of man are pathogenic microbes (e.g., viruses and bacteria). While most of these microbes are larger than 100 nm, their nano-sized components may be shed or released to trigger an immune response of the host. The same applies to nano-sized components shed from even larger organisms, such as invertebrates like house dust mites and cockroaches, which shed chitin nanoparticles from their defoliating exoskeletons. In addition to microbial and zoological sources, naturally occurring nanoparticles include organic and inorganic "dusts" from wildfires, dust storms, and volcanic activity.

The technological ability to manipulate matter at the nanoscale is relatively new. However, physicist Richard Feynman introduced the idea of manipulating individual atoms and molecules in 1959. A decade later, the term "nanotechnology" was coined by Professor Norio Taniguchi. Scientific advances such as the introduction of the scanning tunneling microscope in the early 1980s, capable of visualizing individual atoms, enabled the emergence of modern nanotechnology. The Nobel Prize was awarded to Richard Smalley in 1985 for discovery of the Buckminsterfullerene or "Buckeyball," a C60 carbon nanoparticle. This landmark event was followed by the discovery of the carbon nanotube by Sumio Iijima in 1991. These discoveries set the stage for nanotechnology companies to emerge in the 1990s. Nanotechnology gained momentum by the turn of the twenty-first century when production methods became more controllable and ENMs with adjustable properties were generated, characterized, and imaged at atomic resolution. The National Nanotechology Initiative (NNI) was launched in 2000 to coordinate federal research and development (R&D) efforts and promote US competitiveness in nanotechnology. Similar efforts were launched in the European Union (EU), China, and Japan.

While natural environmental nanoparticles continue to present a challenge to the human immune system and promote immune-mediated diseases, exposure to nanoparticles released as a by-product of anthropogenic activity has been in progress since the Industrial Revolution. Combustion associated with the burning of fossil fuels for energy and transportation contributed to an increase in air pollution particulate matter (PM); a complex mixture of agents (e.g., polycyclic aromatic hydrocarbons and transition metals) surrounding a carbon core. The emergence of new occupational diseases with an immunological basis accompanied the rise of industry. Metallurgy and welding generated metal and metal oxide particles and fumes, while mining released

mineral particles like coal dust or asbestos fibers, resulting in occupational respiratory diseases such as asthma, hypersensitivity pneumonitis, pulmonary fibrosis, and lung cancer (De Vuyst and Camus 2000). Strong epidemiologic evidence linked exposure with disease outcome. Unfortunately, in many cases irreversible disease (e.g., cancer and fibrosis) had already been diagnosed in the population before such associations were confirmed. A noteworthy example is the epidemic of asbestos-related lung diseases: pulmonary fibrosis and mesothelioma. Therefore, disease prevention associated with exposure to products of the emerging nanotechnology industry seems like a logical "lesson learned" and should compel society to avoid repeating mistakes of the past with regard to occupational or consumer exposure and human health. However, advances in nanotechnology are moving forward faster than toxicology research aimed at identifying the relative risk of emerging ENMs. Moreover, the number of products on the market incorporating ENMs will inevitably continue to grow as the number of new applications increases.

1.3 Innate and Adaptive Immune Responses to ENMs

Immunotoxicity can be defined as any adverse effect on the immune system following toxicant exposure. Further, immunotoxic effects can be subdivided into five adverse event categories: (1) immunosuppression, (2) immunogenicity, (3) hypersensitivity, (4) autoimmunity, and (5) adverse immunostimulation (Hussain et al. 2012). *Immunosuppression* refers to impairment of immune cell components leading to decreased immune function, resulting in increased susceptibility to infectious diseases or tumor growth. The other four adverse event categories involve *immunostimulation*. Specifically, *immunogenicity* results in an allergic response following multiple exposures to a foreign agent. Disease outcomes such as asthma and contact dermatitis fall into this category. *Hypersensitivity* involves immune sensitization to a foreign agent that results in a severe adverse response. *Autoimmunity* refers to the immune system adversely reacting to "self" antigens, resulting in tissue destruction by host cells. *Adverse immunostimulation* refers to any antigen-nonspecific activation of the immune system. As illustrated in Fig. 1.1, ENMs may have either immunosuppressive

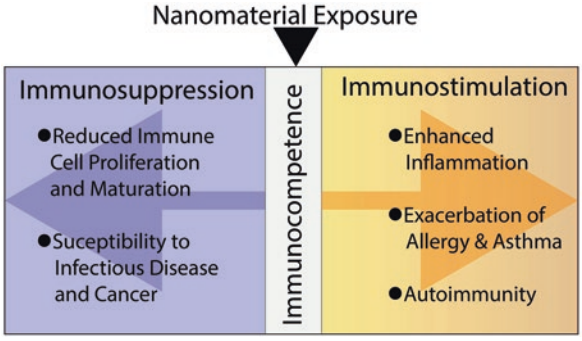

Fig. 1.1 Illustration of the basic principles of immunosuppression and immunostimulation induced by engineered nanomaterials (ENMs)

or immunostimulatory effects. Whether ENMs exert immunosuppressive or immunostimulatory effects likely depends on the timing and context of exposure, as well as susceptibility factors such as age, sex, genetics, co-exposure with other immunogenic agents, or preexisting disease (Scoville et al. 2017; Frank et al. 2017; Duke et al. 2018; Ihrie et al. 2019).

The immune system consists of a network of specific cell types and secreted biomolecules that work in a coordinated fashion to recognize "non-self" materials, communicate the invading threat, and execute defensive mechanisms. Typically, the immune system is divided into the *innate immune system* that rapidly responds to foreign invasion and the delayed but highly specific *adaptive immune system*. The adaptive immune system is endowed with immunological memory following initial response to a specific antigen, leading to an enhanced response to subsequent exposures to the same antigen. This type of immune sensitization results in the polarization and expansion of specific T lymphocyte populations that recognize and react against a specific antigen and is the underlying basis for vaccination used in the prevention of infectious diseases in humans.

Many of the immunotoxic effects of ENMs are mediated via direct interaction with the innate immune system (Farrera and Fadeel 2015). The innate immune system consists of cells in the mononuclear phagocytic system that includes tissue macrophages, peripheral blood monocytes, and granulocytes such as neutrophils. Macrophages, discussed in Chap. 2, represent the earliest response to ENM exposure and function to remove foreign material as well as serve other important functions in host defense (Hussell and Bell 2014). Neutrophils also play important roles in the innate immune response to microbes as well as ENMs and are recruited from the circulation to injured tissues by chemokines released by resident cells, including macrophages (Borregaard 2010). The diverse functions of neutrophils in the immune response to ENMs are discussed in Chap. 3.

Mast cells also play a prominent role in innate immune responses. These cells are abundant in tissues exposed to the external environment (e.g., lung, gastrointestinal tract, and skin) where they are a first responder to insult. Upon activation, they immediately release preformed mediators via degranulation such as histamine, serotonin, proteases, and lipid mediators that promote recruitment of immune cells to the surrounding tissue as well as many physiological effects such as increased vascular permeability and cross talk with the nervous system. Following degranulation, mast cells further produce a myriad of cytokines in a late phase response that further define the local immune response by recruitment of eosinophils, neutrophils, and macrophages, as well as promoting the polarization of T cells. Evidence from cell culture experiments in vitro and rodent studies in vivo indicates that ENMs can trigger mast cell activation and degranulation. For example, mast cells orchestrate adverse pulmonary and cardiovascular responses to carbon nanotubes through the interleukin 33/suppression of tumorigenicity 2 receptor (IL-33/ST2) axis (Katwa et al. 2012). The role of mast cells in mediating immunotoxicity to ENMs is discussed in Chap. 4.

While the innate immune system is the immediate defense against foreign agents, the adaptive immune response takes days or weeks to develop. However, once an

adaptive immune response has been established, the immune system rapidly responds to subsequent exposure to the same foreign agent. Cells of the adaptive immune system include dendritic cells, T cells, and B cells. In general, T cells are involved in cell-mediated responses, while B cells mediate humoral immunity (antibody production). T cells can be further polarized to different T helper (Th) CD4+ lymphocyte subtypes (Th1, Th2, Th17) after antigen presentation by dendritic cells in combination with the appropriate cytokine microenvironment, giving rise to either Th1 "non-allergic" inflammation or Th2/Th17 "allergic" inflammation (Lambrecht and Hammad 2015). Regulatory T cells are required for maintenance of immune tolerance, essential for the discrimination between "self" and "non-self." Dendritic cells play a central role in the immune response by coordinating innate and adaptive immunity and serving as inducers of the adaptive immune response through antigen presentation to T lymphocytes. These cells are effective phagocytes at primary target organs exposed to ENMs (lung and intestine) and further facilitate processing and presentation of foreign antigens to T cells (Banchereau and Steinman 1998). After phagocytosis of the foreign agent, immature dendritic cells migrate from peripheral primary target tissues to lymph nodes, where they undergo maturation and stimulate T cells and B cells.

In general, exposure to foreign agents could lead to immunosuppression, wherein the immune system fails to respond in expanding specific T cell populations, or immunostimulation that potentially leads to autoimmune or allergic disease (Luster 2014; Boraschi et al. 2017). While, fewer studies have been conducted on the effects of ENMs on the adaptive immune response, a few studies indicate that ENMs may disrupt dendritic cell function, which may in turn affect T and B cells. For example, carbon nanotubes have a direct effect on dendritic cells that diminishes their ability to stimulate T cell proliferation in the spleen (Tkach et al. 2011). The role of dendritic cells in ENM-induced immunotoxicity or as a therapeutic target for immune regulation by nanoparticles is discussed in Chap. 5.

1.4 Physicochemical Properties of ENMs that Determine Immunotoxicity

Size is a key physicochemical property that determines the unique behavior of ENMs in biological systems, allowing for translocation across tissue and cellular barriers, as well as interactions with biomolecules and intracellular organelles. *Shape* is also an important feature of ENMs that could determine immunogenicity. ENMs come in a variety of shapes, including spheres, hedrons, fibers, tubes, and sheets. Some examples are shown in Fig. 1.2. For fiber or tube-shaped ENMs, the rigidity of the structure also plays a role in innate immune cell recognition. In addition to size and shape, a variety of other *physicochemical properties* determine the immunotoxic activity of ENMs. For example, some ENMs undergo partial or complete *dissolution* in aqueous fluids and release ions, while other ENMs are relatively stable in solution or suspension. ENMs can also form agglomerates that may modify

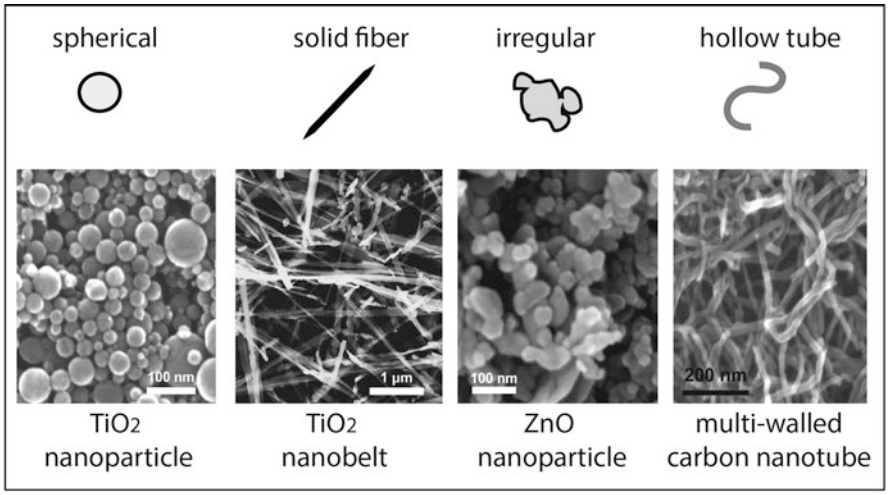

Fig. 1.2 Examples of engineered nanomaterials (Reproduced with permission from Ihrie and Bonner, 2018)

immune cell recognition and uptake or translocation across biological barriers. *Agglomeration* of ENMs, due to noncovalent interaction through van der Waals forces or electrostatic attraction, may result in the formation of micron-sized particles. However, unlike larger particles, ENM agglomerates with the same overall dimensions retain extremely high surface area and reactivity due to the loose attraction between the individual components. The *surface charge* on ENMs, referred to as the *zeta potential*, determines agglomeration as well as other interactions with biomolecules, cell membranes, and translocation across mucosal barriers such as the lining of the lungs or gastrointestinal (GI) tract. Upon interaction with biological systems, some physicochemical characteristics, such as surface charge, change as the ENMs accumulate a biocorona and move across biological barriers (discussed below).

1.5 Biocorona Formation and Recognition of ENMs by the Immune System

In biological systems, proteins, lipids, and carbohydrates adsorb to the surfaces of ENMs to form a *biocorona.* The biocorona coating on the ENM is often what the immune cell first recognizes and therefore is important in mediating these interactions. For example, the composition of the biocorona can determine whether an ENM is recognized by immune cells as biocompatible or immunotoxic. While physicochemical properties determine the synthetic identity of ENMs, the biocorona determines *biological identity*; that is to say, how immune cells recognize

ENMs (Fadeel et al. 2013). Biocorona composition depends on the extracellular microenvironment surrounding the ENM. For example, inhaled ENMs encounter a different makeup of biomolecules in the lungs (e.g., surfactant proteins and lipids) as opposed to ENMs ingested into the GI tract. The formation of the primary biocorona in the respiratory or GI tracts could modify the penetration and passage of nanoparticles across biological barriers from the lungs or intestines into the circulation, although it has been shown that an albumin biocorona around gold nanoparticles does not impede their translocation across the air–blood barrier in the lungs in rats (Konduru et al. 2017). Once in the circulation, a *secondary biocorona* forms through interaction with serum proteins such as albumin, immunoglobulins, and complement proteins. Natural antibodies may recognize epitopes of "self" proteins in the biocorona as "non-self" since proteins are known to undergo denaturation and unfold when bound to nanoparticles (Deng et al. 2011). The effect of ENMs, specifically nanoparticles, on the complement cascade and the implications for immunotoxicity versus nanomedicine applications are discussed in Chap. 6.

1.6 Translocation of ENMs across Biological Barriers

Upon exposure, ENMs directly contact the skin or may encounter the epithelial lining of the respiratory or intestinal tract after inhalation or ingestion, respectively. ENMs have been reported to translocate to different tissues and organs after exposure, whether the exposure route is inhalation, ingestion, or dermal. The keratinized epidermis of the skin generally acts as an effective barrier against ENM penetration, unless there is abrasion or damage resulting from sunburn. In contrast to skin, the epithelial lining of the respiratory and GI tract are more susceptible to injury by ENM exposure and translocation of ENMs can occur much more readily. The ability of ENMs to cross the air–blood barrier in the lung or the gut–blood barrier in the GI tract depends on the size and shape of the nanoparticle, but can also be influenced by surface charge, agglomeration status, and biocorona surrounding the ENM. For example, after inhalation, noncationic nanoparticles with a diameter of less than 34 nm that do not bind serum proteins reach lymph nodes within about 30 min (Choi et al. 2010). Translocation of ENMs across biological barriers to the organs of the immune system (lymph nodes, thymus, spleen) provides direct access to immune cells (Geiser and Kreyling 2010). It is also possible for ENMs with appropriate physicochemical characteristics to accumulate in other organ systems (liver, kidneys, heart), or cross the blood–brain barrier or the placental barrier (Kreyling et al. 2010). It is noteworthy that ENMs do not necessarily have to translocate to influence immune responses in tissues distant from the primary target organ. For example, inhaled carbon nanotubes cause release of soluble signals from the lungs (e.g., TGF-β1) that causes immunosuppression in the spleen (Mitchell et al. 2009). The translocation, bioaccumulation, and fate of ENMs in the body are discussed in Chap. 7.

1.7 Oxidative Stress in the Immune Response to ENMs

Many of the toxicological effects of inhaled particles are due to oxidative stress. For example, the oxidative stress potential of ambient air pollution particles, especially the ultrafine fraction (i.e., nanoparticles), has been attributed at least in part to transition metals (e.g., zinc, copper, and iron) that generate reactive oxygen species (ROS) either via Fenton-like reactions or by stimulating an oxidative burst when engulfed by phagocytes. For example, for nanoparticles that directly generate ROS through surface chemistry, decreasing particle diameter generally correlates with increasing toxicity. This is because an equivalent mass of smaller particles has a greater surface area per unit mass compared to larger particles. The generation of ROS in response to air pollution nanoparticles has been implicated in the pathogenesis of immune-related lung diseases in humans such as asthma and pulmonary fibrosis (Li et al. 2008). Moreover, ROS have been implicated in mediating the toxicity of ENMs in cells and experimental animals (Nel et al. 2009).

Two major mechanisms of ROS generation have been proposed. First, molecular oxygen interacts with the ENM surface, in the absence of cells, to generate free radicals such as superoxide ion and hydroxyl radical. Like ambient air pollution particles, the oxidative potential of metal oxide nanoparticles is due in part to generation of ROS via Fenton-like reactions. Second, ENMs activate inflammatory cells (e.g., macrophages and neutrophils) to stimulate the release of ROS through activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system on cell membranes. For example, NADPH oxidase-derived ROS have been shown to play a central role in pulmonary fibrogenesis induced by exposure to carbon nanotubes (Shvedova et al. 2008). Intracellular ROS can also be generated from the mitochondria via disruption of the electron transport chain and ENMs can also disrupt mitochondrial homeostasis. For example, silver nanoparticles have been reported to reduce adenosine triphosphate (ATP) content in cultured glioblastoma cells and fibroblasts, causing damage to mitochondria and increasing production of ROS (Asharani et al. 2009). To counterbalance ROS generation, cells possess antioxidant systems, including *superoxide dismutase* (SOD), *catalase*, and *glutathione redox systems,* which degrade specific oxidants in specific ways. However, ENMs have the potential to inhibit these antioxidant systems. Finally, ROS can also serve as signaling intermediates to activate intracellular signaling molecules such as receptor tyrosine kinases, mitogen-activated protein (MAP) kinases, and transcription factors, leading to the expression of genes involved in immune responses (Thompson et al. 2014). The role of ROS in ENM-induced immunotoxicity is discussed in Chap. 8.

1.8 ENMs and Immune-Mediated Diseases

Immune-mediated diseases linked to toxicant exposure in the human population include asthma, hypersensitivity pneumonitis, pulmonary fibrosis, allergic contact dermatitis, and autoimmune diseases. It is also increasingly recognized that

cancer has a strong immunological basis and cells of both the innate and adaptive immune system are found in the tumor microenvironment (Gajewski et al. 2013). As discussed earlier in the Sect. 1.2, there is a wealth of epidemiological evidence that links occupational and environmental exposure to toxicants (e.g., ambient particles and metals) (Cooper et al. 2002). However, human disease associated with exposure to ENMs has not been documented. Logically, one could make the assumption that this is either because ENMs will not cause human disease or it is simply too early, given the recent advent of nanotechnology. The latter is likely more plausible than the former, based on adverse effects of metals and the fact that many ENMs are composed of metals or metal oxides. However, epidemiological studies confirm that ENM exposure has occurred in workers (Basinas et al. 2018). Moreover, there is some evidence of adverse inflammatory or immune responses in workers exposed to ENMs. For example, biomarkers of pulmonary fibrosis are increased in workers exposed to multiwalled carbon nanotubes (Fatkhutdinova et al. 2016).

Much of what we know about the potential for ENMs to cause immune-related diseases is inferred from studies with rodents. For example, there is a wealth of information on ENMs in causing pulmonary fibrosis and allergic lung disease in rodents that has been recently reviewed (Duke and Bonner 2018; Ihrie and Bonner 2018). ENMs are also causes of contact dermatitis in experimental animals. The evidence for ENMs in causing or exacerbating airway inflammation and asthma or allergic dermatitis is discussed in Chap. 9.

1.9 ENM Interaction with the Microbial World and Implications for Immunity

In addition to the effects of ENMs on host immune cells, it is also possible that ENMs impact host susceptibility to microbes; bacteria and viruses that constantly pose a challenge to our immune system. It is established that exposure to ambient ultrafine particles by inhalation can enhance host susceptibility to viral and bacterial infections. Some types of ENMs also increased susceptibility to viral infection. For example, single-walled carbon nanotubes (SWCNT) increase infectivity of human epithelial cells exposed to pandemic influenza A and also increase viral titers in mice (Sanpui et al. 2014; Chen et al. 2017). Chapter 10 focuses on the impact of ENMs on host susceptibility to viral infections. While ENMs may increase host susceptibility to microbes, certain types of ENMs could also be exploited as delivery platforms to target viruses or bacteria for eradication. On the other hand, ENMs in the gut could wreak havoc on the host microbiome, which is essential for maintenance of host immunity. For example, dietary silver nanoparticles have been shown to disrupt the gut microbiome in mice (van den Brule et al. 2016).

1.10 Methods for Assessing the Immunotoxicity of ENMs

A tiered approach is commonly used for immunotoxicity testing of chemicals and for the most part, the same principles can theoretically be applied to ENMs. In the first tier, standard toxicity tests (e.g., 90-day repeated dose toxicity study) are performed in rodents to assess systemic effects of ENMs on organs of the immune system after inhalation or oral exposure. In the second tier, more specific immune function assays are utilized; for example, antibody formation or resistance against infectious agents. However, these tiered studies performed in rodents are increasingly impractical given the increasing variety of different ENMs, which is further complicated by postsynthesis surface functionalization to enhance functional properties of the materials. As a result, new and improved in vitro tests and in silico approaches are being developed that address high-throughput immunotoxicity testing, which will reduce reliance on animal testing (Nel et al. 2013). Specifically, state-of-the-art in vitro cell culture systems coupled with relevant dose–response exposure systems are increasingly being improved and refined to assess ENM immunotoxicity at the cellular level (Alépée et al. 2014; Fytianos et al. 2016; Septiadi et al. 2018). These in vitro systems for ENM immunotoxicity testing are discussed in Chap. 11. In addition, novel "organ-on-a-chip" technologies could provide more advanced in vitro assessments of immunotoxicity (Polini et al. 2019). Collectively, these alternative approaches will allow for the comparative analysis of large numbers of ENMs simultaneously and will also be important for hazard assessment at various stages of product development as well as throughout the life cycle of ENMs (Thomas et al. 2009). Finally, it is increasingly apparent that an integrated approach toward assessing the issue of nanoimmunotoxicology will be accomplished by the coordinated efforts of toxicologists, immunologists, engineers, exposure scientists, and risk assessors to foster sustainable development of nanotechnology.

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