



Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

The immune effects of naturally occurring and synthetic nanoparticles

Christopher Chang

Division of Rheumatology, Allergy and Clinical Immunology, University of California at Davis School of Medicine, 451 Health Sciences Drive, Suite 6510, Davis, CA 95616, USA

ABSTRACT

Keywords:

Ultrafine particles
Nanoparticles
Nanotechnology
Air pollution
Climate change
Diesel exhaust particles
Asthma
Anthropogenic
Environmental tobacco smoke
Particulate matter
PM_{2.5}
PM₁₀
Oxidative stress
Adjuvants
Reactive oxygen species
Carbon nanotubes
Autoimmunity

Ultrafine particles and engineered nanoparticles have unique aerodynamic and biochemical properties that affect the immune system and human health in ways that are different from or exceed those seen with gases or larger particulates. These effects result from a unique set of physical characteristics and surface moieties, which generate an ability of UFPs to enter tissues and cells, interact with proteins and DNA at a molecular level and directly and indirectly modulate the immune system by novel mechanisms. In recent years, a new field known as nanotechnology has impacted multiple industries by taking advantage of the special qualities of these small “atomic-sized” particles. Nanomedicine has already opened up a new avenue of research in cancer therapy, drug delivery and immune regulation. While the benefits of this new science to human civilization are seemingly immeasurable, it is also important to appreciate that these particles can also lead to harmful effects on human health. *In vitro* and animal studies are showing that nanoparticles and UFPs are capable of activating proinflammatory cytokines, chemokines and adhesion molecules, with recruitment of inflammatory cells including basophils, macrophages, dendritic cells, T cells, neutrophils and eosinophils. These changes may have an impact on immune defense, but also on the Th1/Th2 balance, and even on non-immunologic function. Resulting immune system derangement can lead to increases in incidence of autoimmune, allergic and even neoplastic diseases. Cardiorespiratory effects have been observed to occur in humans. Much further research is needed to establish safe exposure levels for this important new class of particulates.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Particulate matter is categorized based on size. PM₁₀ represents spherical particles that are 10 µm in diameter or less, or non-spherical particles that have an aerodynamic diameter of 10 µm or less, PM_{2.5} particulates have an aerodynamic diameter of 2.5 µm or less, and UFPs and nanoparticles are defined as particles 100 nm in diameter or

less. In order to understand how particulate pollutants interact with living organisms or tissues, whether to the benefit or detriment of human health, it is important to understand the physical and chemical properties of these entities, and from where they originate. At this length scale, surface area to mass ratio becomes a more critical factor [1], and a consequence of that may be an increase in biological activity due to the favorable presentation of molecularly active moieties on the surface of the particle. This may manifest as a newly discovered or previously unrecognized property resulting from the quantum mechanical nature of these small particles.

Nanoparticles are particles of the same size range as ultrafine particles. It is commonly accepted that the distinction between ultrafine particles and nanoparticles lies in their origin. Ultrafine particles are those particles that are produced and released as spurious emissions resulting from everyday life or industrial byproducts, and are thus classified as air pollutants. In contrast, nanoparticles are engineered, but the distinction between these two groups is not always clear-cut. Thus, particles of nanometer scale can be loosely divided into three categories, those that are naturally occurring (natural UFPs), those that are produced inadvertently as a result of human activity (anthropogenic UFPs), and those that are deliberately engineered for specific uses (nanoparticles) (Table 1).

Abbreviations: 8-OHdG, 8-hydroxy-2-deoxyguanosine; ABC, atmospheric brown cloud; AQI, air quality index; ARE, antioxidant response element; BEC, biliary epithelial cells; CPPs, cell-penetrating peptides; DEP, diesel exhaust particles; FEV1, forced expiratory volume in 1 s; FPG, formamidopyrimidine DNA glycosylase; FVC, forced vital capacity; GFAP, glial fibrillary acidic protein; GI, gastrointestinal; GSH, glutathione; GSSG, glutathione disulfide; HIF-1, hypoxia-inducible factor-1; HO-1, heme-oxygenase-1; LPS, lipopolysaccharide; MDA, malondialdehyde; MP, microprocessor; NEHL, Nanotechnology Environmental and Health Implications; NK, natural killer; NKT, natural killer T cell; Nrf2, NF-E2 related factor 2; NSET, Nano-scale Science Engineering and Technology; OGG1, 7,8-dihydro-8-oxoguanine-DNA glycosylase; PAH, polycyclic aromatic hydrocarbons; PBC, primary biliary cirrhosis; JNK, phosphorylated Jun N-terminal kinase; pJNK, phosphorylated JNK; PM, particulate matter; RANTES, regulated upon activation, normal T cell expressed, and secreted; ROS, reactive oxygen species; TLR, toll like receptor; TNF, tumor necrosis factor; UFP, ultrafine particle.

E-mail address: c3chang@yahoo.com.

Table 1
Nanoparticles.

Naturally occurring ultrafine particles			
Type of particle			
Volcanic ash			
Magnetotactic bacteria			
Ocean spray			
Mineral components			
Anthropogenic ultrafine particles			
Source		Components	
Diesel exhaust particles (DEP)		Polycyclic aromatic hydrocarbons, aldehydes, aliphatic compounds, carbonaceous core [112]	
Environmental Tobacco Smoke (ETS) [67]		Condensed tar particles – alkenes, nitrosamines, aromatic and heterocyclic hydrocarbons and amines [113]	
Engineered nanoparticles			
Particulate species	Approximate size (nm)	Industry	Examples of use
DNA nanotubes	4–20	Medicine	Streptavidin bound on DNA array
Carbon nanotubes	50	Electronic	Photovoltaic cells, transistors
(cylindrical fullerenes)		Materials science	Mountain bike handlebars
		Medicine	Cancer treatment
Fullerenes	1 (for a C60 fullerene)	Materials science	Construction of materials with high tensile strength
Membrane nanotubes		Naturally occurring	May be related to cell–cell transmission in HIV as extension of plasma membrane
Nanowire	1	Electronics	To replace or complement carbon nanotubes, high conductivity material
Nanocoatings		Construction and	Anti-microbial surfaces which are toxic to bacteria and viruses
		Cleaning	Cleaning supplies
Nanolithography	<100	Printing	Fabrication of leading edge integrated circuits, nanoelectromechanical systems
Quantum dots		Electronics	Photovoltaic cells, light emitting devices, solid state quantum computation, organic dyes, imaging of cells, gene silencing therapy
Carbon black		Paint	Pigment
		Automotive	Reinforcement for rubber tires
Colloidal Gold	Submicrometer particles	Electronics	As a drug, as a drug carrier
		Medicine	

2. The health impact of ultrafine particles

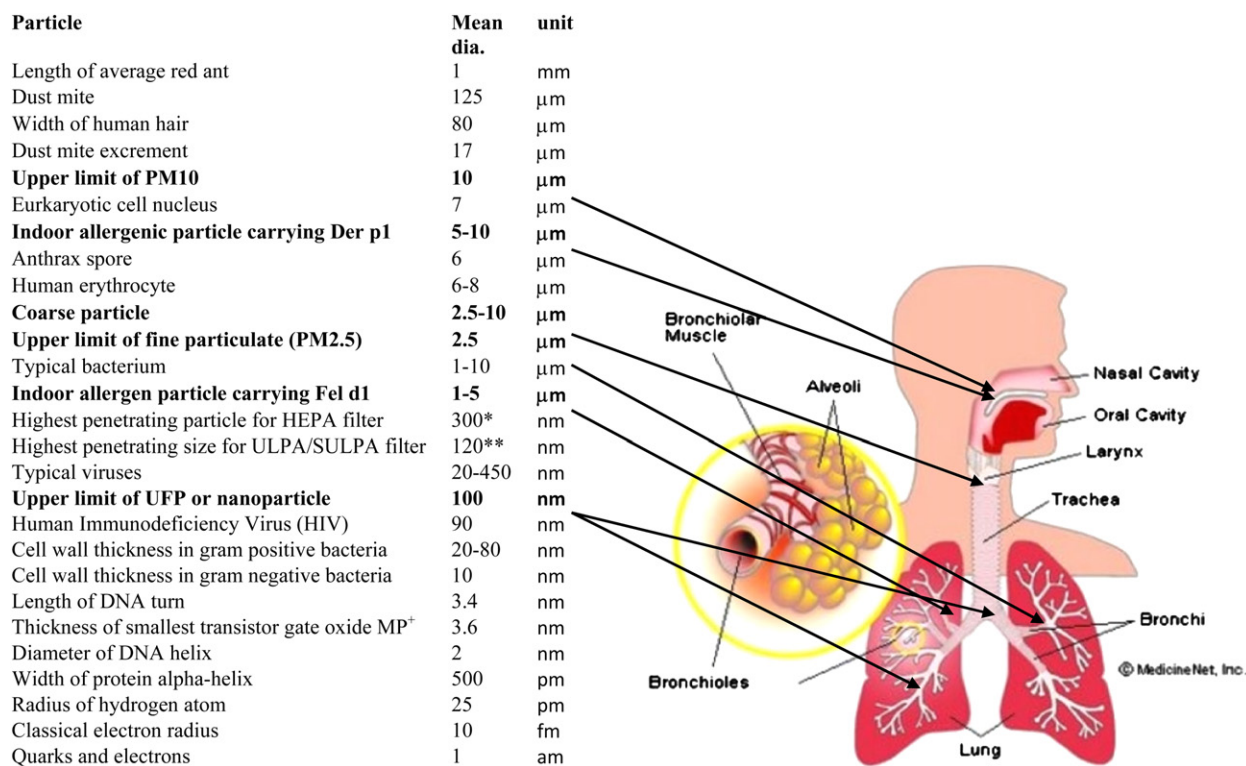
It has been demonstrated that ultrafine particles or nanoparticles are associated with adverse health effects such as asthma and cardiovascular disease. The potential adverse health effects of naturally occurring or anthropogenic ultrafine particles may be either immunologic or non-immunologic in nature. We will review the pro-allergic and pulmonary effects of ambient ultrafine particles and then use this knowledge to improve our understanding of the potential hazards of engineered nanoparticles, extending our discussion to the potential biological interactions that are based on the novel physicochemical properties of these particles. These health effects may or may not be explained by the ultrafine particle paradigm of oxidative stress and inflammation. We will discuss the importance of nanotechnology, and why it is necessary to develop regulatory standards to ensure the safety of exposure to engineered nanoparticles in order to reap the tremendous benefits this new science has to offer mankind. It is important also to realize that in addition to respiratory tract exposure, ultrafine particles also occur in foods and food additives as dietary contaminants of air pollution, and now more increasingly are also used as synthetic agents to change the appearance or texture of foods, such as the use of micro- or nanoparticulate TiO_2 as a whitening or brightening agent [2,3]. The gastrointestinal (GI) tract, like the respiratory system and the skin, is a major immune organ which serves as an interface between host and environment, and the effect of nanoparticles and ultrafine particles of the diet on gut immunity and the role in autoimmune diseases such as Crohn's disease [2] will be discussed as well.

3. Physicochemical characteristics of ultrafine particles

The rapid development of new technologies and industries that bring about the comforts of modern society has been accompanied by an increase in anthropogenic air pollution. These pollutants are generally products of combustion, such as the burning of fuel in

automobile engines and the byproducts of power plants. The result of these changes has been a rapid rise in the quantity and variety of pollutants in the air, including particulate matter pollutants. The impact of air pollutants on human health has been extensively reviewed [4,5]. Particulate air pollutants are of special importance because of their ability to carry a variety of chemicals that are hazardous to human health. Particle size is one of the most important considerations when studying adverse health effects in humans. Particles larger than $10\ \mu\text{m}$ generally do not effectively penetrate and deposit into the lower respiratory tract, and are cleared by nasal and airway ciliary mucosa. In contrast, progressively smaller particles show greater penetration into more distal parts of the respiratory tract (Fig. 1). These particulates have larger surface to mass ratios and are preferentially subject to Brownian movement and diffusion, rather than impaction and gravity. Geometric and mathematical models of nanoparticle movement as a function of airflow convection, dispersion, and axial diffusion have been used to study deposition of these particles in the human lung. Predictive models showed significant variation in deposition depending on asymmetric lung geometrical models [6]. The increased deposition and retention rates of ultrafine particles, along with the observation that there is now a global haze of small particles that exists in the atmosphere over the industrialized northern hemisphere, sometimes referred to as the atmospheric brown cloud (ABC) illustrates the importance of studying the associated adverse health effects.

A study of autopsy specimens of Vancouver lungs demonstrated that smaller particles were found in higher particle loads in the respiratory bronchioles compared with the main stem bronchus. High concentrations were also found at the carinas of large airways. The UFPs were found in singlet arrangement and were interestingly found to consist mostly of metals. Vancouver has a low ambient concentration of ultrafine particle pollutants. In contrast, examination of lungs from Mexico City, where there is a high ambient exposure to particulate pollutants, showed large numbers of aggregates of



* N100 HEPA filter removes 99.97% of particles at 300nm, while N97 HEPA filter removes 95% of particles at 300nm ** Ultra Low Penetration Air (ULPA) filter removes 99.999% of particles at 120nm, and a Super ULPA (SULPA) filter removes up to 99.9999% of particles at 120nm *MP = microprocessor

Fig. 1. Relative sizes of particles relevant to UFPs and nanoparticles and patterns of airway deposition of particles of various sizes.

carbonaceous chains of ultrafine particles in the airway mucosa [7]. It is apparent that where one lives determines the type of ultrafine particle exposure, and its associated dangers.

Jaques and Kim studied 22 healthy volunteers (11 men and 11 women) breathing preparations of ultrafine particles of different size content with 6 different breathing patterns and found that there was higher total deposition fraction with smaller particles. In the case of particles of the smallest size tested (40 nm), the total deposition fraction was significantly higher in women than in men. The reason for this is not clear, but it does demonstrate that multiple factors such as gender may have a subtle influence on how ultrafine particles generate their effects of respiratory function [8]. A study by Nemmar et al. showed that inhalation of a radiolabeled preparation of carbon particles with Technetium-99m (^{99m}Tc) by five healthy volunteers led to detection of radioactivity in the blood within 1 min, reaching a peak between 10 and 20 min and finally clearing within 60 min [9]. The retention of particles in the lower airway may also vary as a result of their size. A study by Moller et al. using Technetium-99m (^{99m}Tc)-radiolabeled 100 nm diameter carbon particles demonstrated 96% retention of the particles after 24 h. This negligible clearance indicates that chronic exposure may lead to higher and higher concentrations accumulating in the lung periphery. Inability to clear ultrafine particles may be a mechanism by which these particles produce more significant health effects. Mucociliary clearance is further impeded in patients who smoke or have chronic obstructive pulmonary disease [10].

4. Sources of ultrafine particles

Sources of ultrafine particles can be categorized into mobile or stationary, and can also be classified as either point, line or area sources. Particulate pollutants can also be classified as primary or

secondary. An example of a primary UFP source is diesel exhaust particles (DEPs) seen in areas of high volume traffic. Other sources of primary UFPs include power plants, environmental tobacco smoke, barbecuing, and cooking and heating in indoor environments. Secondary UFPs can form from the condensation of vapors, including sulfuric and semivolatile organics (Table 2) or from conversion or activation of primary UFPs, leading to the generation of newly or more highly reactive species. Ambient ultrafine particles such as those contained in a sample of DEPs are generally a heterogeneous group of particles of varying sizes, shapes, and chemical and surface characteristics. DEPs have become the model for studies on the immune effects of ultrafine particles. They are made up of a carbonaceous core or a nucleus, which adsorbs on its surface multiple compounds that have the ability to generate proinflammatory effects, including organic chemicals such as polycyclic aromatic hydrocarbons (PAH), metals, nitrates and sulfates (Table 3). These surface compounds can either exert direct effects on inflammatory cells or act in conjunction with an allergen (as an adjuvant). DEPs are comprised of coarse particles (2.5–10 μm), fine particles (PM_{2.5}) and UFPs. It is important to realize that the design and development of so-called clean emission engines may

Table 2

Common sources of ultrafine particulate matter pollution.

Road transport
Power plants
Forest fires
Deliberate biomass burning
Biogenic VOCs
Cooking and Heating
Natural hazards such as volcanic eruptions or earthquakes
Cigarette smoke

Table 3
Components of UFPs.

Carbonaceous core
Surface organic hydrocarbons
Benzoperylene
Benzo(b)fluoranthene
Benzo(e)pyrene
Benzo(a)pyrene
Methyl substituted MW
Coronene
Benzo(k)fluoroanthene
Benz(a)anthracene
Indeno(cd)pyrene
Other minor components
Transition metals
Iron
Copper
Chromium
Titanium
Nickel
Cobalt
Vanadium
Surface nitrates
Surface sulfates

paradoxically contribute to an increase in UFPs, as there are currently no monitoring standards for UFPs. In developing monitoring standards, secondary sources of UFPs may be overlooked, as these will only appear after activation following release into the atmosphere.

The analysis of the immunological and non-immunological effects of ultrafine particles requires the ability to collect concentrated samples of particulates of different sizes distributions. The Versatile Aerosol Concentration Enrichment System (VACES) is a mobile system that has been utilized to obtain samples with a homogeneous set of characteristics (e.g. size). A new electrostatic precipitator system (ESP) operating in tandem with VACES allows for more efficient sampling and the shorter sampling times allow for an increase in viable cell retrieval to facilitate *in vivo* and *in vitro* toxicity studies [11]. The physicochemical properties of these particles can be studied and the effects of ultrafine particles on oxidative stress responses can be measured in biotic and abiotic assays. The dithiothreitol (DTT) assay measures the formation of reactive oxygen species (ROS) by quinone catalysis and is a way to measure *in vitro* ROS generation by particulate matter. It has been demonstrated that UFPs have a higher redox activity than do particles of larger size distribution. As will be discussed below, UFPs also generate increased cellular oxidative stress than FPs or coarse particles [12].

5. Mechanisms of injury of DEPs

5.1. Oxidative stress and DEPs

Particles depositing into various areas of the respiratory tract are either phagocytized by macrophages, cleared in mucus secretions, or may even penetrate airway epithelium and enter the body. Because of the small size of UFPs, they can enter cells by endocytosis and tend to localize inside mitochondria. The effects of ultrafine particles on human health have largely been explained by the presence of ROS on the surface of these particles, but this is not the only mechanism of injury. ROS bind readily to lipids, proteins, DNA and the cellular membranes and produces cellular injury by

overtaxing the protective mechanisms within cells that are normally triggered when exposed to oxidative stress.

Xia et al. describe a three-tier system based on the extent of ROS to create cellular injury coupled with the human body's ability to cope [13]. In Tier One, exposure to ROS leads to activation of the normal protective mechanisms available to living cells which will be discussed below. Tier Two response occurs when the protective mechanism is overwhelmed by the extent of oxidative injury and this leads to activation of transcription factors that generate inflammation, while a Tier Three response describes cytotoxicity at the cellular level. UFPs are potent inducers of cellular heme-oxygenase-1 expression, a marker of oxidative stress. In Tier One responses, normal levels of naturally occurring antioxidants such as intracellular glutathione protect against ROS induced injury. Nrf2 is b-ZIP transcription factor located in the nucleus which interacts with an antioxidant response element (ARE) to generate enzymes with antioxidant properties, and thereby protect against particulate matter derived proinflammatory effects. However, in Tier Two responses, the normal antioxidant level is insufficient to handle the level of ROS injury. UFPs can also lead to depletion of intracellular glutathione [14], thereby fueling their own inflammatory and cytotoxic effects.

Highly reactive surface molecules found on UFPs may include transition metals such as copper, vanadium, chromium, nickel, cobalt and iron, which can promote the generation of oxygen rich intermediates via a reaction known as the Fenton reaction (Fig. 2). In this reaction, the transition metal catalyzes a redox reaction, most probably in the endoplasmic reticulum [15], leading to the production of reactive oxygen species from hydrogen peroxide. It has been demonstrated that the resulting oxygen reactive species may cause DNA damage. However, it is also important to appreciate that reactive oxygen species are not always harmful, and at certain concentrations may actually have a protective effect due to their ability to modulate gene expression of hypoxia-inducible factor-1 [16], a protein that promotes longevity in *Caenorhabditis elegans* [17]. Other components of UFPs include polycyclic aromatic hydrocarbons and polar organic chemicals such as ketones and quinines. Intracellular enzymes in bronchial epithelial cells can convert the polyaromatic carbons to redox cycling quinones by cytochrome P450 1A1. The resulting species can be converted to semiquinones by NADPH cytochrome P450 reductase, which can donate electrons to oxygen to create superoxide moieties (O_2^-).

Because of their role in cellular respiration, energy processing and utilization of oxygen, mitochondria are the most active subcellular sites of ROS production. The rich electron environment of the mitochondria can lead to electron leakage through perturbation, a direct way that reactive oxygen species can be generated. But activation of mitochondrial enzymes such as NADPH oxidase also leads to the generation of large quantities of O_2^- , which is involved in the mechanism by which phagocytes initiate intracellular killing of pathogens. Increased levels of ROS production by inflammatory cells increases methacholine induced airway hyper-responsiveness, and lead to increased levels of exhaled markers of oxidative stress, increased pulmonary extracellular production of superoxide dismutase and glutathione peroxidase and reduced antioxidant levels [18].

The Fenton Reaction



Fig. 2. The Fenton reaction.

5.2. Effects of UFPs on Th1/Th2 balance

Another mechanism by which UFPs produce cellular inflammation involves the modulation of Th2 and Th1 balance through the generation of specific cytokines. Inflammation is associated with activation of MAPK and NF κ B, leading to the expression of proinflammatory cytokines and chemokines (a Tier Two response). DEP exposure can lead to a Th2 paradigm, and a suppression of the Th1 response, as evidenced by a mouse study in which DEP-induced oxidative stress causes an inhibition of TLR-induced dendritic cell maturation and a suppression of IL-12 secretion [19]. Interestingly, DEP alone resulted in a non-specific stimulation of cytokine production and an increased total IgE, but failed to demonstrate active IgE isotype switching as evidence by the lack of deleted switch circular DNA. A subsequent study showed that DEP, when injected before or together with LPS, strongly inhibited the LPS-induced IFN- γ response, while only slightly inhibiting the LPS-induced IL-10 response. The LPS-induced TNF- α response was unchanged. The authors found that DEP was unable to inhibit the IFN- γ response by anti-CD3 mAb-activated NKT cells. They concluded that it is through Toll like receptor (TLR) ligand induction of IFN- γ that DEP is able to promote a Th2 response, which is independent of the effects produced as a result of generation of reactive oxygen species [20].

A 2006 study by Takano et al. investigated the contribution of various parts of the DEP towards the modulation of the immune system and found that different DEP components caused different effects on the immune system. For example, washed DEP, which basically contain only the residual carbonaceous nuclei, when combined with lipopolysaccharide (LPS), was able to increase lung expression of proinflammatory chemokines and cytokines and resulted in exacerbation of lung injury in male ICR mice. On the other hand, the organic chemical component of DEPs resulted in a Th2 shift, characterized by eosinophilic lung inflammation, goblet cell proliferation and enhanced lung expression of IL-5 and eotaxin [21]. DEPs also augmented the LPS stimulation of TLR4 expression [22]. In a mouse study looking at differential effects of DEP components, it was observed that when the carbonaceous nuclei was combined with LPS and instilled into the trachea of mice, the expression of both COX 1 and 2 increased in the lung. On the other hand, combining the OC component of DEPs with LPS led to a decrease in COX2 gene expression compared to LPS alone [23].

5.3. The adjuvant effects of ultrafine particles

UFP can also act as adjuvants to enhance the inflammatory response. Activation of alveolar macrophages causes release of proinflammatory mediators [24,25]. In 2001, Heo et al. showed that either the carbonaceous core of DEPs or the organic extracts, when injected with ovalbumin into mice, were able to augment ovalbumin specific IgG, IgG1 and IgG2a as compared to ovalbumin injected alone [26]. When peripheral blood monocytes from allergic patients were exposed to either DEP-PAHs or Der p1 separately, there was an increase in chemokines IL-8, RANTES as well as TNF- α , but a synergistic effect was noted with simultaneous exposure of DEP-PAH and Der p1 [27]. An *in vitro* study of the effects of a mixture of polyaromatic hydrocarbons derived from DEPs on peripheral blood mononuclear cells (PBMCs) extracted from healthy subjects revealed that DEP-PAHs increase secretion of IL-8 and CCL5, but inhibit release of MCP-1 in a dose dependent manner [28]. This effect appeared to be modulated at the transcriptional level, as production levels of mRNA for IL-8, CCL5 and MCP-1 paralleled the protein secretion. Intranasal exposure to combined ovalbumin and carbon black particles also led to an increase in CD4+ T cell proliferation in peribronchial lymph nodes, which was

significantly greater than that seen by exposure to ovalbumin alone [29]. Intranasal instillation of DEPs and ragweed pollen in humans led to an increased production of ragweed specific IgE and IgG₄. Increases in mRNA levels for the Th2 cytokines interleukins IL-4, 5, 10 and 13 with a concomitant reduction in expression of interferon- γ was observed [30,31]. Exposure of DEPs and soluble egg antigen to intestinal mucosa led to an enhanced systemic Th1 and Th2 response in mice [32]. The effect of ultrafine carbon black particles on the migration and maturation of myeloid dendritic cells *in vitro* showed an upregulation of CD80/CD86 expression after exposure to ovalbumin plus carbon black particles. There was an associated increase in cytokine expression, including IL-4, IL-5, IL-10, IL-13 and interferon- γ . Interestingly, there was no proliferation or activation of plasmacytoid dendritic cells (pDC), which are thought to induce tolerance via the airways. The mechanisms of action of UFPs and nanoparticles are illustrated in Fig. 3.

5.4. Autoimmunity and UFPs

While most of the research on the adverse immune effects of UFPs and nanoparticles has focused on the importance of a Th2 cytokine milieu on the development of allergic diseases, there is sufficient evidence that under the right conditions, these particles can generate predominantly Th1 effects and lead to autoimmune diseases as well [33–36]. Autoimmunity is a result of an interaction between genetic, environmental and epigenetic factors [37–41]. Numerous genes may have a role in development of autoimmunity, and may influence regulation of apoptosis, tolerogenicity, and other potential mechanisms [42]. Environmental exposures may play a role as well, an example being the development of autoimmune thyroiditis after exposure to pollutants such as polyaromatic hydrocarbons that may be carried on UFPs [43]. ETS exposure has also been associated with autoimmune thyroid disease [44].

Evidence of a link between autoimmune diabetes and air pollution was demonstrated in a mouse study, in which the rate of developing frank diabetes was measured in NOD mice exposed twice weekly to aerosolized EHC-93 particles (ambient urban particulate matter). It was observed that EHC-93 exposed mice had higher titers of anti-insulin antibodies, and accelerated the onset of diabetes, although the cumulative incidence of diabetes was unchanged [33].

N-hexadecane, a component of DEP, injected intraperitoneally into normal Balb/c mice led to the production of lupus-related autoantibodies [45]. Diesel exhaust particles have been associated with a higher risk of collagen-induced arthritis in mice [46,47]. Particulates have been shown to stimulate both Th1 and Th2 cytokine release [32], but what determines the ultimate skewing may lie in the presence or absence of appropriate cofactors [48]. The gastrointestinal tract is itself a major immune organ and it has been shown that particulate matter, including ultrafine particles, is processed differently in the GI tract compared to soluble antigens. When processed by antigen presenting cells, particulates are able to stimulate MHC class II restricted T cell hybrids with a 5-fold greater efficiency compared to soluble antigen [49]. Alternatively, macrophages may also present ultrafine particles in the context of an alternative MHC Class I pathway, leading to increased proliferation of CD8+ T cells. With regard to synthetic nanoparticles, many food additives contain large amounts of particles in this size range with active highly charged surface molecules, and this may lead to increase in adsorption of luminal or environmental antigens [49].

Other mechanisms may play a role. The link between oxidative stress and cell damage, subsequently leading to cell death and either apoptosis or unprogrammed cell death is an intriguing one in the search for the pathogenesis of autoimmunity. Apoptosis is a highly regulated mechanism, one in which clearance of cell debris

Immune and non-immune effects of UFPs and nanoparticles

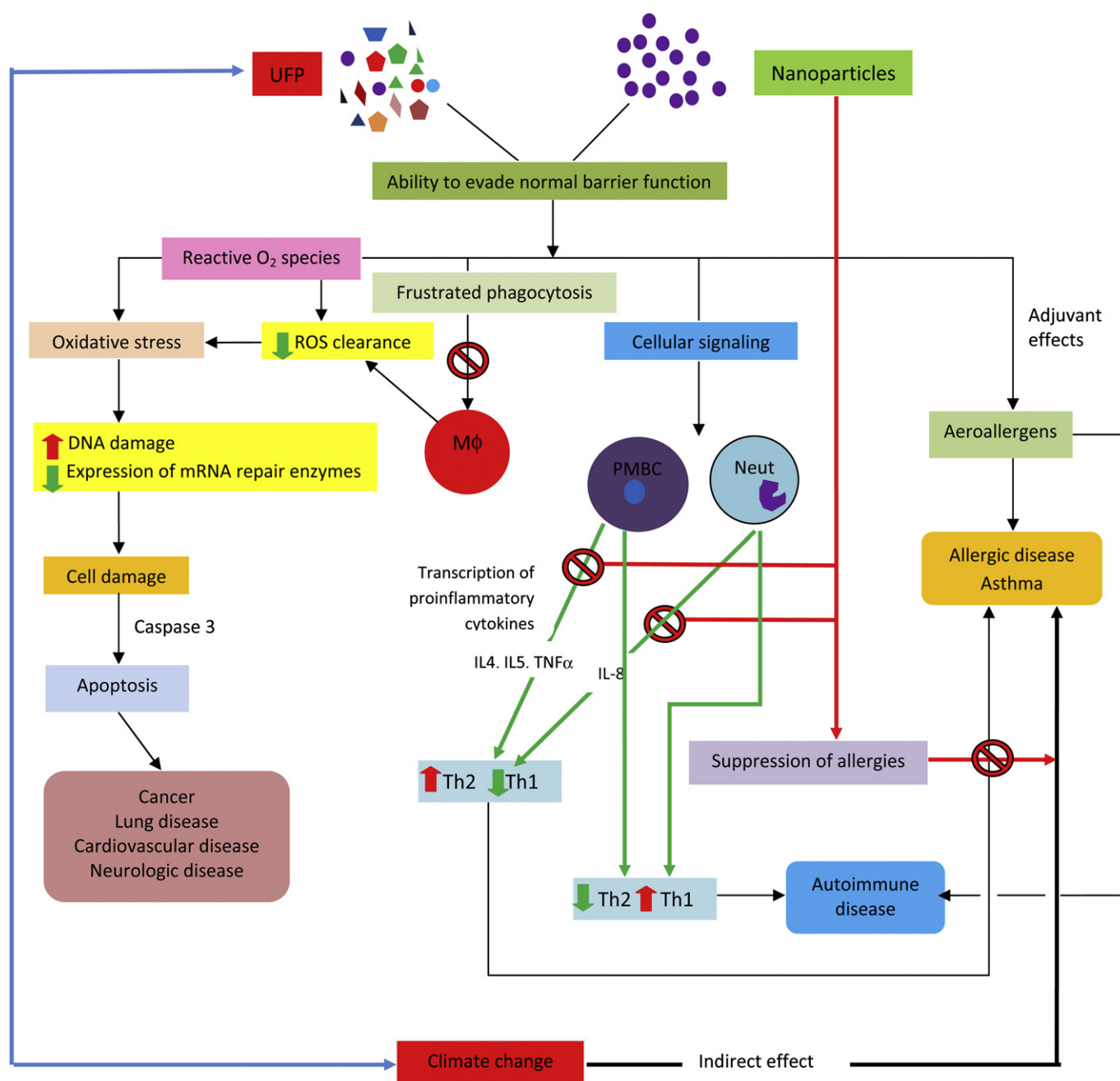


Fig. 3. Immune and non-immune effects of UFPs and nanoparticles. UFPs and nanoparticles exert their effects on a variety of cells and biological processes. The effects of these particles on cellular signaling can either be stimulatory, or in the case of some synthetic nanoparticles, suppressive, leading to either exacerbation of inflammatory processes such as allergic disease or asthma, or potentially a mechanism to treat these diseases, respectively. UFPs also have the potential, by virtue of their effects on climate change, to influence the prevalence and severity of allergic diseases such as asthma and allergic rhinoconjunctivitis.

is done in a controlled fashion with efficient processing by phagocytic cells and prevention of release of potentially highly antigenic material as targets for the development of antibodies [50]. Impairment in apoptosis has been suggested as a potential contributing cause of autoimmunity [51]. Normally functioning Fas and Fas ligand interactions are important in clearance of immunocompetent effector cells, and aberrations in this system has been implicated in the development of autoimmunity [52].

Oxidative stress induced apoptosis has been shown to be factor in the biliary damage of primary biliary cirrhosis (PBC). Reduced

levels of glutathione-S-transferase and the production of hypochlorous acid can occur as a result of oxidative stress from exposure to UFPs [53]. High levels of exposure can lead to a Tier 2 or Tier 3 response which overwhelms the process of normal apoptosis. HOCl has been shown to induce apoptosis in biliary epithelial cells (BEC) and increase activity of caspase-3 and expression of pJNK and p53, mechanisms that have postulated to play a role in the generation of UFP induced cellular damage. In dyslipidemic ApoE^{-/-} mice exposed to diesel exhaust particles, oxidatively damaged DNA and liver inflammation was detected [54].

In addition to their role as adjuvants in allergic diseases, air pollutants may also trigger an immune response to normally innocuous or self-antigens. Airborne particulate matter, including UFPs, may stimulate bone marrow leading to a systemic inflammatory response, which results in the release of inflammatory cells, including monocytes [55] and proinflammatory cytokines or co-stimulators [56]. The release of these proinflammatory cytokines by diesel exhaust particles and the resulting oxidative stress have been shown to lead to the maturation of dendritic cells [57–59]. Downstream effects may include enhanced antigen presentation by airway dendritic cells. This enhanced presentation of antigen by highly activated antigen presenting cells (APC) along with the appropriate costimulatory molecules may be able to generate peripheral tolerance in self-reactive T cells that have failed to develop central tolerance in the thymus, contributing to the development of autoimmunity [60]. Systems genetics analysis for genetic variants may be beneficial to understanding the genetic interactions within the thymus, and to potentially identify those who are at risk for environmentally triggered autoimmunity [61].

These are several potential mechanisms by which UFPs may lead to autoimmunity. It is likely that the pathogenesis of UFP induced autoimmunity is multifactorial, with multiple mechanisms playing a role. Retrospective case control studies have shown a relationship between the early onset of type 1 diabetes and exposure to ozone or particulate air pollution [62], as well as a relationship between children with type 1 diabetes and exposure to cigarette smoke, ozone and sulfate [63]. A higher risk of lupus or rheumatic disease was also observed in a community exposed to elevated levels of airborne petroleum products and mercury [64]. A study of dietary microparticle intake in patients with Crohn's disease showed that those patients who took a "low-microparticle diet" for four months had a reduction in Crohn's disease activity index [2]. The effect of a conjugate consisting of ultrafine TiO₂ bound to LPS on peripheral blood mononuclear cells was conducted on 28 patients with ulcerative colitis, 21 patients with Crohn's disease and 36 controls. The results indicated that the conjugate was able to stimulate peripheral blood mononuclear cells to secrete interleukin-1 (IL-1) 30–60-fold above that observed in cells not incubated with the conjugate. The increase appeared to be greater in patients with inflammatory bowel disease, and there was minimal stimulation with TiO₂ or LPS alone. Moreover, intestinal (colonic) biopsy specimens from these subjects cultured with TiO₂-LPS conjugate led to a 2–3-fold increase in IL-1 secretion compared to cultures with TiO₂ or LPS alone. The data supported a role of ultrafine dietary particles in overcoming normal intestinal cell tolerance to endogenous molecules as a contributing factor in autoimmune gastrointestinal disease [65].

5.5. Pulmonary inflammation and cardiopulmonary system injury

A comparative study on the effects of exposure in mice to ultrafine particles (<0.18 µm), fine particles (<2.5 µm) and filtered air was conducted using a mobile toxicology inhalation laboratory, in which the Apolipoprotein E-deficient mice (genetically susceptible to atherosclerosis), were exposed to concentrated ambient particle mixes in whole-body exposure chambers [66]. Mice exposed to an environment with a higher content of UFPs experience a higher number and larger size of atherosclerotic plaques than those mice in the other two chambers. The authors attribute this to the higher concentration of UFPs in the UFP chamber. It was also proposed that it was the higher propensity for UFP to stimulate oxidative stress that led to the development of plaques. An increase in liver lipid peroxidation was found in both the UFP and the FP exposed mice, which led to an increase in phase II antioxidant responses mediated through Nrf2, as well as higher levels of

glutathione 2-transferase Ya, catalase, superoxide dismutase and NADPH-quinone oxidoreductase. Also observed were higher hepatic malondialdehyde (MDA) levels in the UFP exposed mice. MDA is a marker of oxidative stress. The anti-inflammatory property of HDL, measured by the number of migrated monocytes, was diminished in the UFP group as well. The level of exposure of FP and UFP in this study was estimated to be about 2–6 times that which we would normally expect in humans sitting in cars on freeways.

5.6. Non-inflammatory mechanisms of cardiac injury

Because of their small size, UFPs can also penetrate directly into the blood and lymphatic circulation by transcytosis or endocytosis, and thereby can gain access to multiple organs including heart, spleen, lymph nodes and bone marrow. Many of the ROS on the surface of these particles can directly induce DNA strand breaks and lead to cardiac injury via a non-immunologic mechanism [67]. DEPs exposure can lead to altered heart rate which is thought to be a direct effect on pacemaker activity [68]. While the mechanism for the enhancement of thrombosis associated with DEP instillation into hamster lungs may be immune-related [69], direct translocation of DEPs into the circulation may also be a contributing factor [70,71]. It has also been demonstrated that DEPs can induce platelet aggregation in the hepatic microvasculature as a result of prothrombotic changes that are not related to inflammatory reactions [72].

5.7. Effects on the nervous system

There is also evidence that UFP exposure can induce activation of the immune system in the brains of mice. This was shown by an increase in expression of the transcription factors NFκB and AP-1, as well as an increase in glial fibrillary acidic protein (GFAP) after particulate inhalation. Activation of JNK was also noted at lower exposure concentrations of ultrafine particles [73]. The systemic inflammation resulting from exposure to ultrafine particles produces oxidative stress that may also play a role in neurodegeneration [74].

6. What is known about the cardiorespiratory effects of UFPs in humans?

A summary of current data from *in vitro*, animal and human studies of exposure to DEP, DEP and allergen together, ultrafine particles and nanoparticles is shown in Table 4. Holgate et al. exposed human subjects to a high ambient concentration (300 µg/m³) of diesel exhaust for 1 h. Findings included a marked neutrophilic response in the airways and increases in peripheral blood neutrophil and platelet counts. There was upregulation of IL-8 and increase in expression of IL-8 mRNA. Clinical effects seen in control and asthma subjects exposed to 100 µg/m³ of diesel exhaust for 2 h included an increase in airway resistance in both groups. An increase in IL-10 staining in biopsy specimens of airway mucosa was seen in subjects with asthma. P-selectin expression was noted to be upregulated in bronchial biopsy specimens from control subjects [75].

Twenty-nine healthy adults were exposed to either UFPs or filtered air, in conjunction with exercise in a cross over study to determine the effects on oxidative damage to DNA and the capacity to repair such damage. The exposure was controlled and designed to simulate real-life ambient air particles, and physical exercise was also employed to simulate real-life conditions in which the ventilation rate and thereby the extent of exposure is increased during exercise. The air in the exposure chamber was monitored for particle size distribution and numbers, as well as concentration of

Table 4
Immunological and clinical effects of ultrafine particles.

Particulate preparation	<i>In vitro</i> studies	Animal studies	Clinical or epidemiologic studies in humans
DEP	• Inhibition of LPS-induced dendritic cell responses • Increased chemokine production	• Proliferation of goblet cells • Enhanced lung expression of chemokines and cytokines with proinflammatory activity • Increase expression of IFN-γ	• Increase in airway resistance, increase in neutrophils in bronchial wash in control subjects, lack of effect in mild asthmatics
DEP with allergen	• Synergistic increased in chemokine and MAP kinase activation	• DEP with LPS enhanced COX2 gene expression in mice • DEP with ovalbumin increased allergen specific IgE and IgG1 levels in mice	• Enhanced allergen specific IgE and Th2 skewing • Nasal challenge leads to <i>in vivo</i> IgE isotype switching
UFP	• Increased chemokine and cytokine generation (IL-4, IL-5, IL-8 IL-10, IL-13, interferon-γ) • Increased adhesion molecule expression • Direct effects on neutrophils, mast cells and eosinophils • Effects on epithelial cells via proinflammatory mediators • Increased cytokine mRNA expression in respiratory tract mucosa • Increased migration and maturation of myeloid dendritic cells in lung tissue • IgE isotype switching in humans (Th2 switching) • Increased goblet cell hyperplasia with exposure to OC on surface of UFPs • Inhibition of allergic responses • Induction of oxidative stress and mitochondrial damage • Increased DNA breaks and oxidative stress	• Increased systemic oxidative stress and early atherosclerosis in mice • Increased JNK activation and CNS inflammation in mice	• No effect on pulmonary function, airway NO production
Nanoparticle	• Increased oxidative stress and apoptosis of BEAS-2B cells • Induction of cellular damage and micronucleus formation in V79 cells	• Pulmonary fibrosis and lung tumor in rats • Reduced lung function and increased lung function when administered in conjunction with LPS	

gases. Peripheral blood mononuclear cells were isolated from the subjects and analyzed for DNA strand breaks, activity of 7,8-dihydro-8-oxoguanine-DNA glycosylase (OGG1) and oxidized purines as formamidopyrimidine DNA glycolase (FPG) sites. Exposure to UFP for 6 h and for 24 h was associated with an increased number of DNA breaks. Exercise caused a further increase but did not reach statistical significance. Types of DNA breaks included frank breaks, alkaline labile sites and transient repair breaks. mRNA expression of repair enzymes was not found to be upregulated by exposure to UFPs [76].

A study of 14 asthmatics and 17 controls exposed in a controlled environmental chamber to a concentrated sample of ultrafine particles collected from Los Angeles freeways demonstrated subtle changes in pulmonary function and cardiac parameters, including a 0.5% drop in arterial blood oxygen saturation as measured by pulse oximetry and a 2% drop in morning FEV1 the day after exposure. There was also subtle evidence of autonomic cardiac function disruption in that heart rate variability was noted to be reduced on holter monitoring. These observations occurred in both groups of subjects. Unfortunately this was a small study and the results were not convincing, and further large-scale studies may be needed to determine if the clinical effects observed in this study truly exist [77]. Brugge et al. reviewed the existing literature to determine if there was sufficient evidence of cardiac and pulmonary health risks as a function of exposure to freeway pollutants [78]. They determined that UFP, black carbon, nitrogen oxides and carbon monoxide levels are all elevated in proximity to highways (freeways) that carry greater than 30,000 vehicles per day. They analyzed the rates of respiratory disease, cardiovascular health effects and cancer as a result of living near highways and found that most of these studies have demonstrated the strongest correlation

between cardiovascular health outcomes and exposure to particulate matter. Living near high volume freeways is also associated with a higher prevalence of wheezing or asthma, and reduced lung function or slow development of lung function has also been observed. Reduction in FEV1 and FVC was also noted to occur in 19 asthmatic children who were wearing personal PM monitors [79].

Studies on the development of asthma suggest that early childhood exposure may be an important factor in determining susceptibility to highway pollutants. Interestingly, girls seemed to be more likely to develop asthma as a result of highway pollutant exposure. Data from the Six-Cities Study and the American Cancer Society Study from the mid 1990s and their accompanying follow-up studies done in the early 2000s demonstrated a link between particulate matter exposure and lung cancer, although there are significant confounding factors [80,81]. The observation of DNA damage from exposure to UFPs discussed above provides further support for the hypotheses of a link between DEP exposure and cancer. These studies provide strong if not completely conclusive evidence of the link between human health and UFP exposure. However, UFP exposure in humans is complex, and it is clear that further studies are important to define clinical effects so that we may establish standards for safe levels of UFP exposure.

7. Regulatory and therapeutic considerations in terms of the health impacts of UFP

Multiple regulatory agencies have defined guidelines for exposure limits for particulate matter PM_{2.5} and PM₁₀. The World Health Organizations 2005 global update on air quality guidelines are 10 $\mu\text{g}/\text{m}^3$ annual mean concentration and 25 $\mu\text{g}/\text{m}^3$ 24 h mean concentrations for PM_{2.5} and double those limits for PM₁₀. In

September 2006, the US Environmental Protection Agency (EPA) tightened the 24-h $\text{PM}_{2.5}$ standard from $65 \mu\text{g}/\text{m}^3$ to $35 \mu\text{g}/\text{m}^3$. The United States EPA currently also defines safety standards as $15 \mu\text{g}/\text{m}^3$ annual mean $\text{PM}_{2.5}$ concentration, and a 24 h mean concentration of $150 \mu\text{g}/\text{m}^3$ for PM_{10} . Similar standards have been defined by the State of California, which has slightly more strict guidelines than the USEPA, the European Union and Canada. These standards are shown in Table 5. In the United States, once the EPA has established a new National Ambient Air Quality Standard (NAAQS), it will then identify and designate areas in which standards have been met (attainment), or not met (nonattainment), or in which there is insufficient data to determine if standards have been met (unclassifiable). Designations are based on three years of air quality monitoring data. As of August 2008, the EPA has notified 25 states that they currently meet fine particle standards. It is important to note that published safety limits for $\text{PM}_{2.5}$ and PM_{10} are either lacking, obsolete or currently being formulated in many other countries, especially in Asia, where air pollution is an immense problem.

Assuming that monitoring programs have been or will be developed, the positive impact on human health will only be realized if one takes the next logical step of implementing procedures that will effectively reduce exposure to harmful particulates. These standard measures involve the reduction in emission of anthropogenic pollutants encountered in industrial plants and factories, traffic emissions and other sources. The long term benefits of a monitoring program make take years to come to fruition.

At the present time there are no regulatory guidelines for “safe” (or “hazardous”) level of exposure to UFPs. These standards are clearly necessary, but it is important to realize that in establishing such standards, one cannot simply utilize or modify existing FP standards. This is because UFPs and “engineered” nanoparticles may have very different immune and non-immune health effects based on the unique properties associated with their size and surface characteristics, as described above. Since we are spending more and more time indoors, it is important to be aware that UFPs may originate indoors as we develop new materials for cleaning, disinfecting and energy conservation. Additionally, UFPs that are generated in outdoor environments may be better able to penetrate into indoor environments because they can escape removal by standard HEPA filters. UFPs have the potential to exacerbate indoor and outdoor allergen associated diseases by their ability to act as adjuvants [82]. Further research needs to be done in order to establish “safe exposure limits”. However, in the meantime, the public awareness of global warming and its relationship to air pollution has fueled public policy so that California has prohibited construction or placement of new schools within 500 feet of a major freeway. Whether or not measures such as these are able to effectively reduce cumulative exposure to UFPs over the long run is yet to be determined. It is also important to take into consideration the rapid development occurring in various global regions, including Asia and South America, which are now increasingly

important in defining the global landscape of air pollution sources [83]. These changes will need to be accounted for in developing models for high exposure areas, and high exposure populations, since weather patterns also contribute to the dispersion of these particles. When developing future guidelines for ultrafine particle exposure safety standards, it will be important to take these global changes into consideration.

8. Ultrafine particles, climate change and seasonal allergies

In addition to the above-mentioned direct effects of UFPs on human health, including their properties of adjuvants leading to the potential exacerbation of allergic asthma, allergic rhinoconjunctivitis and atopic dermatitis, UFPs may exert their influence indirectly in a rather unexpected way that is just now becoming evident. It is evident that our planet is experiencing changes in climate, including global warming and changes in precipitation patterns. The International Panel on Climate Change (IPCC) estimates that there has been an increase in temperature of approximately 2.4°C over preindustrial era levels [84]. This increase has been attributed to anthropogenic sources, and while other factors may coexist, it is likely that emission of greenhouse gases, and the role of particulate matter in the production of atmospheric brown clouds [85] contribute significantly to climate change. The relative magnitude of the role of UFPs or nanoparticles on climate change is unclear, but maintaining the current trend will inevitably induce changes in vegetation over the long run which will potentially impact the incidence of allergic diseases in future years [86]. Interestingly, the reverse process may also become evident. If climate change continues unchecked, the resultant effects, including acceleration of the melting of the polar ice caps, rainfall and change in land mass/water interfaces may lead to the unexpected release of significant amounts of UFP components into the atmosphere or water supply. Thus climate change, a product of air pollution, may in fact itself lead to higher levels of air pollution [87]. This has the potential to create a vicious cycle leading to progressively higher rates of allergic or inflammatory diseases that are known to be associated with ultrafine particle exposure, as discussed above.

9. What is nanotechnology and why must we be concerned about the safety effects of nanotechnology?

Nanotechnology is a relatively new field of technology that takes advantage of the unique properties of particles in the “nano” size range. Nanotechnology is now a major part of almost every industry, including food science, genetic engineering, cosmetics, applied physics, device physics, health, textile, materials science, interface and colloid science, as well as chemical, mechanical, biological, and electrical engineering. Various new sub-disciplines have arisen in recent years, including nanomedicine, nanolithography, DNA nanotechnology, molecular nanotechnology, molecular electronics, and nanorobotics. Types of nanoparticles include nanoclusters, nanopowders, and nanocrystals. Nanoparticles may exist as a single entity, or can aggregate. They have been studied in the context of drug design, potentially as a method for delivery of drugs to reach molecular targets or penetrate normally intact physiologic barriers by virtue of their size characteristics. Some examples of the use of nanotechnology in medicine are shown in Table 6. New imaging methodology that is based on the supramagnetic properties of nanoparticles is being developed. Nanoparticles can be conjugated with ligands such as monoclonal antibodies for more tissue specific targeting [88]. The interaction between nanoparticles and the cell membrane is of particular interest because of applications in drug delivery and targeting [89],

Table 5
Safety standards for non-particulate and particulate pollutants.

Type of particle	WHO	USEPA	California	EU	Canada
Mean annual concentrations					
PM_{10}	25	Revoked	20	40	70
$\text{PM}_{2.5}$	10	15	12	25	
UFP	None	None	None	None	
Mean 24-h concentrations					
PM_{10}	50	150	50	50	120
$\text{PM}_{2.5}$	20	35	None	None	
UFP	None	None	None	None	

Table 6
Medical uses of nanotechnology.

Specialty	Type of technology Function	Particles used or process	Rationale or possible mechanism of action
Pharmacology	Drug synthesis Drug delivery	Lipid based Polymer based Nanoshells Carbon nanotubes Nanofiltration Nanocrystals	1. Takes advantage of ability of nanoparticles to penetrate around and through cells 2. Change in volume of distribution due to better targeting of drug 3. Reduced side effects 4. Altered clearance of drug 5. Combination of drugs with macromolecules 6. Purification and sterilization of drug to remove harmful contaminants
Surgery	Nano-welding	Gold coated nanoshells	1. To seal or fuse tissues in order to control bleeding during surgery
Neurology	Neuro-electronics	Nanoelectronics-based sensors	1. Control of nerve impulses by external computers for treatment of paraplegia
Oncology	Drug delivery Diagnosis Selective killing of tumor cells	Sensor test nanochips Fluorescent quantum dots Dendrimer	1. Increased surface to volume ratio may improve binding to tumor cells 2. Designer nanoparticles for multifunctional uses 3. Carbon nanotubes may be heated with local effects only 4. Photodynamic therapy can be used to selectively heat nanoparticles
Radiology	Imaging Bioimaging	Quantum dots Nanorobots	1. Used in conjunction with MRI imaging to improve images 2. Increase contrast of nanoparticles 3. Targeting nanoparticles to desired sites of action
Immunology Infectious diseases	Vaccine design Allergen immunotherapy	Nanoemerging vaccines Adjuvants	1. Purification of vaccines 2. Photokilling of bacteria 3. Methyl vinyl ether and maleic anhydride nanoparticles
Genetics	Cell repair Gene delivery	Nanomachines	1. Nanoparticles can open and close cell membranes and travel into cells, and can repair DNA at the molecular level
Nephrology	Diagnosis and management of kidney disease	Nanoscale artificial kidney	1. Nanoscale robots will be able to execute curative and reconstructive processes in live subjects
Nutrition	Food science	TiO ₂ as food coloring Food packaging nanoparticles Homogenation Milling Coatings	1. Whitening agent 2. Nanoparticles can be used to kill microorganisms 3. Food packaging with nanomaterials for preservation of food

phototherapy [90], biomedical imaging tools [91] and gene delivery [92]. Cell membrane penetration by synthetic nanoparticles is dependent on electrical charge and the ability to disrupt the phospholipid bilayer without destroying the cell. Other mechanisms of cell entry include disruption of endolysosomal membranes or chaperoning of nanoparticles by cell-penetrating peptides (CPPs). Nanoparticles can also be trapped inside endosomes and thereby never come into contact with cytosol based regulatory factors. Design of nanoparticles with cell-penetrating ability involves synthetic arrangement of surface coatings, such as alternating anionic and hydrophobic groups.

The potential benefits are far-reaching, but as with any new technology, one must be cognizant of the inevitable side effects. Nanoparticles of substances traditionally believed to be inert, such as titanium dioxide, may take on a different set of reactive properties. Adverse effects of engineered nanoparticles may be easier to study than ambient UFPs because the former tend to be more homogeneous in shape, size, and surface characteristics. At this time, there are no human studies to show that nanoparticles produce human disease, but there is experimental data to indicate that there may be potential hazards. For example, when cultured human lung epithelial cells BEAS-2B were treated with cerium oxide nanoparticles ranging from 15 to 45 nm, a number of cytotoxic effects were observed, including increase in reactive oxygen species, reduction in glutathione levels and the induction of HO-1, catalase, glutathione-S-transferase and thioredoxin reductase, all markers of oxidative stress. Production of cytosolic caspase-3 and chromatin condensation was observed, ultimately leading to apoptosis [93]. In an animal study of photocatalytic titanium dioxide (TiO₂) nanoparticles, intraperitoneal injections of

nanoparticles followed by challenge with aerosolized ovalbumin was found to promote a predominant Th2 response with elevated IgE and IgG1 and infiltration of neutrophils, eosinophils and lymphocytes into the bronchoalveolar fluid [94]. A similar finding was reported in mice that were challenged with ovalbumin in the presence of single and multiple walled carbon nanotubes, whereupon they developed an allergic immune response characterized by an increase in ovalbumin specific IgE, eosinophilic infiltration and release of Th2 cytokines [95].

Surface characteristics may also play a role in induction of cellular damage, as shown in a study comparing vanadium pentoxide-coated ultrafine titanium dioxide particles and uncoated titanium dioxide particles. The study showed that the former were much more reactive and had a greater ability to induce DNA damage in V79 hamster lung fibroblasts [96]. Another study looking at the effect of particle size on toxicity revealed that nanoparticle preparations of titanium dioxide had an increased effect on IL-8 mRNA expression, IL-8 protein release and glutathione (GSH) depletion compared with the same mass of fine particle titanium dioxide. The authors suggested that when evaluating toxic levels of nanoparticles, surface area may be a better parameter to use than mass [97]. Lung tumors and pulmonary fibrosis in rats exposed to ultrafine preparations of TiO₂ have been described. Increased mutation frequency has also been described in cultured human lymphoblastoid cells exposed to ultrafine TiO₂ particles [98]. TiO₂ nanoparticles have been shown to be cytotoxic in mouse fibroblast cells by a mechanism thought to be consistent with oxidative stress, as levels of ROS were observed to increase with increasing concentrations of TiO₂ [99]. Other studies have confirmed the *in vitro* effects of titanium

dioxide on cultured human bronchial epithelial cells, with exposure to TiO₂ nanoparticles leading to increased apoptosis, increased oxidative stress, and reduced expression of glutathione. The release of multiple proinflammatory cytokines including IL-1, IL-6, IL-8, TNF- α and CXCL2 was seen. The observed localization of TiO₂ nanoparticles inside the cell in a perinuclear pattern may shed some light on how the behavior of nanoparticles differs from larger size fractions of particulates [100].

An example of the “two-edged sword” nature of new technologies such as nanotechnology is the use of titanium dioxide nanocoatings in hospitals and other institutions as a means of disinfection. The theory is that a thin layer of nanoparticles of titanium dioxide can be applied as a coating to walls and other surfaces. Applications of this characteristic include self-cleaning glass and solar cells. Titanium dioxide in its normal state has low-solubility and has not been linked to any significant toxicity. As nanoparticles, titanium dioxide generates oxygen reactive intermediates that are toxic to viruses and bacteria, thereby exerting its intended function. But these particles may also have similar effects on humans. Multiple studies have been done which demonstrate that particulates that are normally considered to be low-solubility, low-toxicity when prepared in the fine particle range take on a significantly different set of toxicity characteristics when generated as nanoparticles (see above). Titanium dioxide and other nanoparticles are also being used as additives to food [101], sun-creams [102,103], and tanning lotions, as well as in the treatment of water pollution [104], and their benefits are boundless [105], but it is important to appreciate that the same unique effects that we are trying to harness to provide benefit for the human race may at the same time produce harmful effects.

Zinc oxide nanoparticles are components of pigments and UV absorbers in personal care products, coatings and paints. They are present in powders or solutions, and can be adsorbed on the skin or inhaled. Inhalation of finely dispersed particulates in metal fumes, especially zinc and magnesium oxide particles results in a flu like illness with symptoms of fever, chills, arthralgias, headaches, fatigue, nausea and muscle aches. Respiratory symptoms mimicking bronchitis have been reported, and the illness resolves several days after removal of the inciting fume. Exposure to nanoparticles in paint sprayers may be responsible for Ardystyl syndrome, a cryptogenic organizing pneumonia described in textile paint workers exposed to polycationic paint components [106]. Carbon nanotubes, which are cylindrical fullerenes used in photovoltaic cells, other electronic components and materials science, are long fibers of nanoscale thickness that are similar in shape to asbestos fibers [107]. The shape of these particles leads to a condition known as frustrated phagocytosis, which is the inability of macrophages to completely engulf a foreign substance [108]. Clearance from lung tissue is thus impaired and this leads to prolonged cellular exposure to ROS and a higher production of inflammatory mediators such as TNF- α [109].

Exposure to nanoparticles is not always proinflammatory. For example, fullerene nanomaterials were found to function as radical scavengers and thereby reduce oxidative stress [110]. The authors reported that antigen-challenged cells that were pre-incubated with fullerene nanoparticles showed a significant inhibition of mast cell and basophil mediator release, with a concomitant reduction in tyrosine phosphorylation of Syk, and an inhibition of IgE-mediated cytoplasmic ROS levels. The fact that fullerenes were able to inhibit features of anaphylaxis in a mouse model, including histamine release and a fall in body temperature suggests a potential therapeutic role in the treatment of allergic diseases. Alternatively, nanoparticles can also be used as a vehicle to deliver negative signaling molecules that may inhibit allergic or inflammatory responses, opening a new avenue

for treatment of asthma, heart disease, multiple sclerosis, rheumatoid arthritis and cancer.

10. Standards for safe levels of exposure for synthetic nanoparticles

From a regulatory standpoint, the EPA is currently in the process of formulating research needed to help understand the risks of exposure to nanoparticles. These risks may or not be similar to those seen with ambient ultrafine particles because of the more homogeneous nature of preparations of nanoparticles. A report released in 2006 outlined the need for research directed at assessing exposure to nanoparticles, the chemical components of nanoparticles and the properties and effects of nanoparticles in biological systems. In addition, the report also addressed the need for studies related to ecologic effects of nanoparticles on the environment, and the potential health effects of nanoparticles. The report also recommends the study of sampling methods for nanoparticles and the development of low-emitting nanomaterials (http://nano.gov/NNI_EHS_research_needs.pdf). It would also be important to define exposure safety limits with regard to how these tiny particles may gain entry into the human body, e.g. contact, ingestion or inhalation. The development of safety guidelines should take into account the physical, chemical and biophysiological properties of nanoparticles and their constituents, because these characteristics may produce varying levels of biological activity on living systems [111]. The resulting screening methods and regulatory guidelines should therefore be developed based not only on size, but also shape and composition.

11. Summary

Nanotechnology is one of the most intensely studied areas of the early 21st century. The development of nanotechnology signifies a revolutionary change in modern science and engineering that promises to re-design the world that we live in. Its wide ranging potential will impact medicine, environmental science and other industries. Already, nanotechnology is being used in the development of treatments for cancer, in construction of ultra-strong materials, and also, ironically, in cleaning up the disasters of the previous industrial revolution, including pollution, chemical toxicity and global warming. However, the resultant release of nanoparticles may present significant potential health effects related primarily to the respiratory, cardiac, and neurologic systems, as has been demonstrated to be the case with anthropogenic UFP exposure. UFP exposure has been associated with an increase in allergies, asthma and autoimmune diseases. However, there are currently no clearly defined safe levels for exposure to ultrafine or nanoparticles, and there is of yet scant solid evidence upon which to base such regulatory standards. Any new technology carries both the hope of the future and the fear of the unknown. Like other revolutionary breakthroughs in science, our goal should be to learn as much as possible about the physical and biochemical nature of nanotechnology so that we can embrace the potential benefits, while at the same time, manage and control any adverse health effects that may result from exposure to these engineered particles.

Acknowledgements

The author would like to thank Drs. Andre Nel and Jonathan Bernstein for their advice and guidance in the preparation of this manuscript.

References

- [1] Schmid O, Moller W, Semmler-Behnke M, Ferron GA, Karg E, Lipka J, et al. Dosimetry and toxicology of inhaled ultrafine particles. *Biomarkers* 2009;14 (Suppl. 1):67–73.
- [2] Lomer MC, Thompson RP, Powell JJ. Fine and ultrafine particles of the diet: influence on the mucosal immune response and association with Crohn's disease. *Proc Nutr Soc* 2002;61:123–30.
- [3] Lomer MC, Thompson RP, Commisio J, Keen CL, Powell JJ. Determination of titanium dioxide in foods using inductively coupled plasma optical emission spectrometry. *Analyst* 2000;125:2339–43.
- [4] Bernstein JA, Alexis N, Barnes C, Bernstein IL, Bernstein JA, Nel A, et al. Health effects of air pollution. *J Allergy Clin Immunol* 2004;114:1116–23.
- [5] Borchers AT, Chang C, Keen CL, Gershwin ME. Airborne environmental injuries and human health. *Clin Rev Allergy Immunol* 2006;31:1–101.
- [6] Asgharian B, Price OT. Deposition of ultrafine (nano) particles in the human lung. *Inhal Toxicol* 2007;19:1045–54.
- [7] Churg A, Brauer M. Ambient atmospheric particles in the airways of human lungs. *Ultrastruct Pathol* 2000;24:353–61.
- [8] Jacques PA, Kim CS. Measurement of total lung deposition of inhaled ultrafine particles in healthy men and women. *Inhal Toxicol* 2000;12:715–31.
- [9] Nemmar A, Hoet PH, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, et al. Passage of inhaled particles into the blood circulation in humans. *Circulation* 2002;105:411–4.
- [10] Moller W, Felten K, Sommerer K, Scheuch G, Meyer P, et al. Deposition, retention, and translocation of ultrafine particles from the central airways and lung periphery. *Am J Respir Crit Care Med* 2008;177:426–32.
- [11] Ning Z, Sillanpaa M, Pakbin P, Sioutas C. Field evaluation of a new particle concentrator-electrostatic precipitator system for measuring chemical and toxicological properties of particulate matter. *Part Fibre Toxicol* 2008;5:15.
- [12] Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, et al. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environ Health Perspect* 2003;111:455–60.
- [13] Xia T, Kovochich M, Nel A. The role of reactive oxygen species and oxidative stress in mediating particulate matter injury. *Clin Occup Environ Med* 2006;5:817–36.
- [14] Li N, Hao M, Phalen RF, Hinds WC, Nel AE. Particulate air pollutants and asthma. A paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clin Immunol* 2003;109:250–65.
- [15] Liu Q, Berchner-Pfannschmidt U, Moller U, Brecht M, Wotzlaw C, Acker H, et al. A Fenton reaction at the endoplasmic reticulum is involved in the redox control of hypoxia-inducible gene expression. *Proc Natl Acad Sci U S A* 2004;101:4302–7.
- [16] Gillespie MN, Pastukh V, Ruchko MV. Oxidative DNA modifications in hypoxic signaling. *Ann N Y Acad Sci* 2009;1177:140–50.
- [17] Zhang Y, Shao Z, Zhai Z, Shen C, Powell-Coffman JA. The HIF-1 hypoxia-inducible factor modulates lifespan in *C. elegans*. *PLoS One* 2009;4:e6348.
- [18] Dong CC, Yin XJ, Ma JY, Millicchia L, Wu ZX, Barger MW, et al. Effect of diesel exhaust particles on allergic reactions and airway responsiveness in ovalbumin-sensitized brown Norway rats. *Toxicol Sci* 2005;88:202–12.
- [19] Chan RC, Wang M, Li N, Yanagisawa Y, Onoe K, Lee JJ, et al. Pro-oxidative diesel exhaust particle chemicals inhibit LPS-induced dendritic cell responses involved in T-helper differentiation. *J Allergy Clin Immunol* 2006;118:455–65.
- [20] Finkelman FD, Yang M, Orekhova T, Clyne E, Bernstein J, Whitekus M, et al. Diesel exhaust particles suppress in vivo IFN-gamma production by inhibiting cytokine effects on NK and NKT cells. *J Immunol* 2004;172:3808–13.
- [21] Yanagisawa R, Takano H, Inoue KI, Ichinose T, Sadakane K, Yoshino S, et al. Components of diesel exhaust particles differentially affect Th1/Th2 response in a murine model of allergic airway inflammation. *Clin Exp Allergy* 2006;36:386–95.
- [22] Inoue K, Takano H, Yanagisawa R, Hirano S, Ichinose T, Shimada A, et al. The role of toll-like receptor 4 in airway inflammation induced by diesel exhaust particles. *Arch Toxicol* 2006;80:275–9.
- [23] Inoue K, Takano H, Yanagisawa R, Ichinose T, Sadakane K, Yoshino S, et al. Components of diesel exhaust particles differentially affect lung expression of cyclooxygenase-2 related to bacterial endotoxin. *J Appl Toxicol* 2004;24:415–8.
- [24] Granum B, Gaarder PI, Lovik M. IgE adjuvant effect caused by particles – immediate and delayed effects. *Toxicology* 2001;156:149–59.
- [25] Takano H, Yoshikawa T, Ichinose T, Miyabara Y, Imaoka K, Sagai M. Diesel exhaust particles enhance antigen-induced airway inflammation and local cytokine expression in mice. *Am J Respir Crit Care Med* 1997;156:36–42.
- [26] Heo Y, Saxon A, Hankinson O. Effect of diesel exhaust particles and their components on the allergen-specific IgE and IgG1 response in mice. *Toxicology* 2001;159:143–58.
- [27] Fahy O, Hammad H, Senechal S, Pestel J, Tonnel AB, Wallaert B, et al. Synergistic effect of diesel organic extracts and allergen Der p 1 on the release of chemokines by peripheral blood mononuclear cells from allergic subjects: involvement of the map kinase pathway. *Am J Respir Cell Mol Biol* 2000;23:247–54.
- [28] Fahy O, Tscopoulos A, Hammad H, Pestel J, Tonnel AB, Wallaert B. Effects of diesel organic extracts on chemokine production by peripheral blood mononuclear cells. *J Allergy Clin Immunol* 1999;103:1115–24.
- [29] de Haar C, Kool M, Hassing I, Bol M, Lambrecht BN, Pieters R. Lung dendritic cells are stimulated by ultrafine particles and play a key role in particle adjuvant activity. *J Allergy Clin Immunol* 2008;121:1246–54.
- [30] Diaz-Sanchez D, Tsien A, Casillas A, Dotson AR, Saxon A. Enhanced nasal cytokine production in human beings after in vivo challenge with diesel exhaust particles. *J Allergy Clin Immunol* 1996;98:114–23.
- [31] Diaz-Sanchez D, Tsien A, Fleming J, Saxon A. Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. *J Immunol* 1997;158:2406–13.
- [32] Yoshino S, Sagai M. Induction of systemic Th1 and Th2 immune responses by oral administration of soluble antigen and diesel exhaust particles. *Cell Immunol* 1999;192:72–8.
- [33] Ritz SA. Air pollution as a potential contributor to the 'epidemic' of autoimmune disease. *Med Hypotheses* 2009.
- [34] van Zijverden M, Granum B. Adjuvant activity of particulate pollutants in different mouse models. *Toxicology* 2000;152:69–77.
- [35] van Zijverden M, van der Pijl A, Bol M, van Pinxteren FA, de Haar C, Penninks AH, et al. Diesel exhaust, carbon black, and silica particles display distinct Th1/Th2 modulating activity. *Toxicol Appl Pharmacol* 2000;168:131–9.
- [36] Yoshino S, Hayashi H, Taneda S, Takano H, Sagai M, Mori Y. Effects of diesel exhaust particle extracts on Th1 and Th2 immune responses in mice. *Int J Immunopathol Pharmacol* 2002;15:13–8.
- [37] Shoenfeld Y, Gilburd B, Abu-Shakra M, Amital H, Barzilai O, Berkun Y, et al. The mosaic of autoimmunity: genetic factors involved in autoimmune diseases – 2008. *Isr Med Assoc J* 2008;10:3–7.
- [38] Shoenfeld Y, Zandman-Goddard G, Stojanovich L, Cutolo M, Amital H, Levy Y, et al. The mosaic of autoimmunity: hormonal and environmental factors involved in autoimmune diseases – 2008. *Isr Med Assoc J* 2008;10:8–12.
- [39] Hewagama A, Richardson B. The genetics and epigenetics of autoimmune diseases. *J Autoimmun* 2009;33:3–11.
- [40] Sanchez-Pernaute O, Ospelt C, Neidhart M, Gay S. Epigenetic clues to rheumatoid arthritis. *J Autoimmun* 2008;30:12–20.
- [41] Invernizzi P. Future directions in genetic for autoimmune diseases. *J Autoimmun* 2009;33:1–2.
- [42] Mackay IR. Clustering and commonalities among autoimmune diseases. *J Autoimmun* 2009.
- [43] Burek CL, Talar MV. Environmental triggers of autoimmune thyroiditis. *J Autoimmun* 2009.
- [44] Tomer Y, Huber A. The etiology of autoimmune thyroid disease: a story of genes and environment. *J Autoimmun* 2009;32:231–9.
- [45] Kuroda Y, Ono N, Akaogi J, Nacionales DC, Yamasaki Y, Barker TT, et al. Induction of lupus-related specific autoantibodies by non-specific inflammation caused by an intraperitoneal injection of n-hexadecane in BALB/c mice. *Toxicology* 2006;218:186–96.
- [46] Yoshino S, Hayashi H, Taneda S, Sagai M, Mori Y. Effect of diesel exhaust particle extracts on collagen-induced arthritis in mice. *Autoimmunity* 2002;35:57–61.
- [47] Yoshino S, Sagai M. Enhancement of collagen-induced arthritis in mice by diesel exhaust particles. *J Pharmacol Exp Ther* 1999;290:524–9.
- [48] Sedlik C, Deriaud E, Leclerc C. Lack of Th1 or Th2 polarization of CD4+ T cell response induced by particulate antigen targeted to phagocytic cells. *Int Immunol* 1997;9:91–103.
- [49] Vidard L, Kovacs-Bankowski M, Kraeft SK, Chen LB, Benacerraf B, Rock KL. Analysis of MHC class II presentation of particulate antigens of B lymphocytes. *J Immunol* 1996;156:2809–18.
- [50] Lleo A, Selmi C, Invernizzi P, Podda M, Gershwin ME. The consequences of apoptosis in autoimmunity. *J Autoimmun* 2008;31:257–62.
- [51] Mackay IR, Leskovsek NV, Rose NR. Cell damage and autoimmunity: a critical appraisal. *J Autoimmun* 2008;30:5–11.
- [52] Stranges PB, Watson J, Cooper CJ, Choisy-Rossi CM, Stonebraker AC, Beighton RA, et al. Elimination of antigen-presenting cells and autoreactive T cells by Fas contributes to prevention of autoimmunity. *Immunity* 2007;26:629–41.
- [53] Salunga TL, Cui ZG, Shimoda S, Zheng HC, Nomoto K, Kondo T, et al. Oxidative stress-induced apoptosis of bile duct cells in primary biliary cirrhosis. *J Autoimmun* 2007;29:78–86.
- [54] Folkman JK, Risom L, Hansen CS, Loft S, Moller P. Oxidatively damaged DNA and inflammation in the liver of dyslipidemic ApoE–/– mice exposed to diesel exhaust particles. *Toxicology* 2007;237:134–44.
- [55] Goto Y, Ishii H, Hogg JC, Shih CH, Yatera K, Vincent R, et al. Particulate matter air pollution stimulates monocyte release from the bone marrow. *Am J Respir Crit Care Med* 2004;170:891–7.
- [56] Becker S, Soukup J. Coarse(PM(2.5–10)), fine(PM(2.5)), and ultrafine air pollution particles induce/increase immune costimulatory receptors on human blood-derived monocytes but not on alveolar macrophages. *J Toxicol Environ Health A* 2003;66:847–59.
- [57] Verstraeten S, Van Den Heuvel R, Nelissen I, Witters H, Verheyen G, Schoeters G. Flow cytometric characterisation of antigen presenting dendritic cells after in vitro exposure to diesel exhaust particles. *Toxicol Vitro* 2005;19:903–7.
- [58] Bleck B, Tse DB, Jaspers I, Curotto de Lafaille MA, Reibman J. Diesel exhaust particle-exposed human bronchial epithelial cells induce dendritic cell maturation. *J Immunol* 2006;176:7431–7.

- [59] Kantengwa S, Jornot L, Devenoges C, Nicod LP. Superoxide anions induce the maturation of human dendritic cells. *Am J Respir Crit Care Med* 2003;167:431–7.
- [60] Poletaev AB, Stepanyuk VL, Gershwin ME. Integrating immunity: the immunculus and self-reactivity. *J Autoimmun* 2008;30:68–73.
- [61] Morahan G, Peeva V, Mehta M, Williams R. Systems genetics can provide new insights in to immune regulation and autoimmunity. *J Autoimmun* 2008;31:233–6.
- [62] Hathout EH, Beeson WL, Nahab F, Rabadi A, Thomas W, Mace JW. Role of exposure to air pollutants in the development of type 1 diabetes before and after 5 yr of age. *Pediatr Diabetes* 2002;3:184–8.
- [63] Hathout EH, Beeson WL, Ischander M, Rao R, Mace JW. Air pollution and type 1 diabetes in children. *Pediatr Diabetes* 2006;7:81–7.
- [64] Dahlgren J, Takhar H, Anderson-Mahoney P, Kotlerman J, Tarr J, Warshaw R. Cluster of systemic lupus erythematosus (SLE) associated with an oil field waste site: a cross sectional study. *Environ Health* 2007;6:8.
- [65] Powell JJ, Harvey RS, Ashwood P, Wolstencroft R, Gershwin ME, Thompson RP. Immune potentiation of ultrafine dietary particles in normal subjects and patients with inflammatory bowel disease. *J Autoimmun* 2000;14:99–105.
- [66] Araujo JA, Barajas B, Kleinman M, Wang X, Bennett BJ, Gong KW, et al. Ambient particulate pollutants in the ultrafine range promote early atherosclerosis and systemic oxidative stress. *Circ Res* 2008;102:589–96.
- [67] Vinzents PS, Moller P, Sorensen M, Knudsen LE, Hertel O, Jensen FP, et al. Personal exposure to ultrafine particles and oxidative DNA damage. *Environ Health Perspect* 2005;113:1485–90.
- [68] Campen MJ, McDonald JD, Gigliotti AP, Seilkop SK, Reed MD, Benson JM. Cardiovascular effects of inhaled diesel exhaust in spontaneously hypertensive rats. *Cardiovasc Toxicol* 2003;3:353–61.
- [69] Lucking AJ, Lundback M, Mills NL, Faratian D, Barath SL, Pourazar J, et al. Diesel exhaust inhalation increases thrombus formation in man. *Eur Heart J* 2008;29:3043–51.
- [70] Nemmar A, Nemery B, Hoet PH, Vermeylen J, Hoylaerts MF. Pulmonary inflammation and thrombogenicity caused by diesel particles in hamsters: role of histamine. *Am J Respir Crit Care Med* 2003;168:1366–72.
- [71] Nemmar A, Hoet PH, Dinsdale D, Vermeylen J, Hoylaerts MF, Nemery B. Diesel exhaust particles in lung acutely enhance experimental peripheral thrombosis. *Circulation* 2003;107:1202–8.
- [72] Khandoga A, Stampfl A, Takenaka S, Schulz H, Radykewicz R, Kreyling W, et al. Ultrafine particles exert prothrombotic but not inflammatory effects on the hepatic microcirculation in healthy mice in vivo. *Circulation* 2004;109:1320–5.
- [73] Kleinman MT, Araujo JA, Nel A, Sioutas C, Campbell A, Cong PQ, et al. Inhaled ultrafine particulate matter affects CNS inflammatory processes and may act via MAP kinase signaling pathways. *Toxicol Lett* 2008;178:127–30.
- [74] Gomez-Mejiba SE, Zhai Z, Akram H, Pye QN, Hensley K, Kurien BT, et al. Inhalation of environmental stressors & chronic inflammation: autoimmunity and neurodegeneration. *Mutat Res* 2009;674:62–72.
- [75] Holgate ST, Sandstrom T, Frew AJ, Stenfor N, Nordenhall C, Salvi S, et al. Health effects of acute exposure to air pollution. Part I: healthy and asthmatic subjects exposed to diesel exhaust. *Res Rep Health Eff Inst* 2003;1–30. discussion 51–67.
- [76] Brauner EV, Forchhammer L, Moller P, Simonsen J, Glasius M, Wahlin P, et al. Exposure to ultrafine particles from ambient air and oxidative stress-induced DNA damage. *Environ Health Perspect* 2007;115:1177–82.
- [77] Gong Jr H, Linn WS, Clark KW, Anderson KR, Sioutas C, Alexis NE, et al. Exposures of healthy and asthmatic volunteers to concentrated ambient ultrafine particles in Los Angeles. *Inhal Toxicol* 2008;20:533–45.
- [78] Brugge D, Durant JL, Rioux C. Near-highway pollutants in motor vehicle exhaust: a review of epidemiologic evidence of cardiac and pulmonary health risks. *Environ Health* 2007;6:23.
- [79] Delfino RJ, Quintana PJ, Floro J, Gastanaga VM, Samimi BS, Kleinman MT, et al. Association of FEV1 in asthmatic children with personal and microenvironmental exposure to airborne particulate matter. *Environ Health Perspect* 2004;112:932–41.
- [80] Dockery DW, Pope 3rd CA, Xu X, Spengler JD, Ware JH, Fay ME, et al. An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 1993;329:1753–9.
- [81] Pope 3rd CA, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, et al. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *JAMA* 2002;287:1132–41.
- [82] Bartra J, Mullol J, del Cuervo A, Davila I, Ferrer M, Jauregui I, et al. Air pollution and allergens. *J Investig Allergol Clin Immunol* 2007;17(Suppl. 2):3–8.
- [83] Siddiqi TA. Asia's changing role in global climate change. *Ann N Y Acad Sci* 2008;1140:22–30.
- [84] Ramanathan V, Feng Y. On avoiding dangerous anthropogenic interference with the climate system: formidable challenges ahead. *Proc Natl Acad Sci U S A* 2008;105:14245–50.
- [85] Carmichael GR, Adhikary B, Kulkarni S, D'Allura A, Tang Y, Streets D, et al. Asian aerosols: current and year 2030 distributions and implications to human health and regional climate change. *Environ Sci Technol* 2009;43:5811–7.
- [86] Shea KM, Truckner RT, Weber RW, Peden DB. Climate change and allergic disease. *J Allergy Clin Immunol* 2008;122:443–53. quiz 454–5.
- [87] Noyes PD, McElwee MK, Miller HD, Clark BW, Van Tiem LA, Walcott KC, et al. The toxicology of climate change: environmental contaminants in a warming world. *Environ Int* 2009;35:971–86.
- [88] Dinauer N, Balthasar S, Weber C, Kreuter J, Langer K, von Briesen H. Selective targeting of antibody-conjugated nanoparticles to leukemic cells and primary T-lymphocytes. *Biomaterials* 2005;26:5898–906.
- [89] Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. *Science* 2004;303:1818–22.
- [90] West JL, Halas NJ. Engineered nanomaterials for biophotonics applications: improving sensing, imaging, and therapeutics. *Annu Rev Biomed Eng* 2003;5:285–92.
- [91] Nahrendorf M, Jaffer FA, Kelly KA, Sosnovik DE, Aikawa E, Libby P, et al. Noninvasive vascular cell adhesion molecule-1 imaging identifies inflammatory activation of cells in atherosclerosis. *Circulation* 2006;114:1504–11.
- [92] Dai H, Jiang X, Tan GC, Chen Y, Torbenson M, Leong KW, et al. Chitosan-DNA nanoparticles delivered by intrabiliary infusion enhance liver-targeted gene delivery. *Int J Nanomed* 2006;1:507–22.
- [93] Park EJ, Choi J, Park YK, Park K. Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells. *Toxicology* 2008;245:90–100.
- [94] Larsen ST, Roursgaard M, Jensen KA, Nielsen GD. Nano titanium dioxide particles promote allergic sensitization and lung inflammation in mice. *Basic Clin Pharmacol Toxicol* 2009.
- [95] Nygaard UC, Hansen JS, Samuelsen M, Alberg T, Marioara CD, Lovik M. Single-walled and multi-walled carbon nanotubes promote allergic immune responses in mice. *Toxicol Sci* 2009;109:113–23.
- [96] Bhattacharya K, Cramer H, Albrecht C, Schins R, Rahman Q, Zimmermann U, et al. Vanadium pentoxide-coated ultrafine titanium dioxide particles induce cellular damage and micronucleus formation in V79 cells. *J Toxicol Environ Health A* 2008;71:976–80.
- [97] Monteiller C, Tran L, MacNee W, Faux S, Jones A, Miller B, et al. The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells in vitro: the role of surface area. *Occup Environ Med* 2007;64:609–15.
- [98] Wang JJ, Sanderson BJ, Wang H. Cyto- and genotoxicity of ultrafine TiO₂ particles in cultured human lymphoblastoid cells. *Mutat Res* 2007;628:99–106.
- [99] Jin CY, Zhu BS, Wang XF, Lu QH. Cytotoxicity of titanium dioxide nanoparticles in mouse fibroblast cells. *Chem Res Toxicol* 2008;21:1871–7.
- [100] Park EJ, Yi J, Chung KH, Ryu DY, Choi J, Park K. Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells. *Toxicol Lett* 2008;180:222–9.
- [101] Augustin MA, Sanguansri P. Chapter 5 nanostructured materials in the food industry. *Adv Food Nutr Res* 2009;58:183–213.
- [102] Wokovich A, Tyner K, Doub W, Sadrieh N, Buhse LF. Particle size determination of sunscreens formulated with various forms of titanium dioxide. *Drug Dev Ind Pharm* 2009;35:1180–9.
- [103] Tyner KM, Wokovich AM, Doub WH, Buhse LF, Sung LP, Watson SS, et al. Comparing methods for detecting and characterizing metal oxide nanoparticles in unmodified commercial sunscreens. *Nanomed* 2009;4:145–59.
- [104] Kim J, Shan W, Davies SH, Baumann MJ, Masten SJ, Tarabara VV. Interactions of aqueous NOM with nanoscale TiO₂: implications for ceramic membrane filtration—ozonation hybrid process. *Environ Sci Technol* 2009;43:5488–94.
- [105] Sandhiya S, Dkhar SA, Surendiran A. Emerging trends of nanomedicine – an overview. *Fundam Clin Pharmacol* 2009;23:263–9.
- [106] Hoet PH, Gilissen LP, Leyva M, Nemery B. In vitro cytotoxicity of textile paint components linked to the “Ardystil syndrome” *Toxicol Sci* 1999;52:209–16.
- [107] Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WA, Seaton A, et al. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nat Nanotechnol* 2008;3:423–8.
- [108] Bainton DF, Takemura R, Stenberg PE, Werb Z. Rapid fragmentation and reorganization of Golgi membranes during frustrated phagocytosis of immobile immune complexes by macrophages. *Am J Pathol* 1989;134:15–26.
- [109] Brown DM, Hutchison L, Donaldson K, Stone V. The effects of PM₁₀ particles and oxidative stress on macrophages and lung epithelial cells: modulating effects of calcium-signaling antagonists. *Am J Physiol Lung Cell Mol Physiol* 2007;292:L1444–51.
- [110] Ryan JJ, Bateman HR, Stover A, Gomez G, Norton SK, Zhao W, et al. Fullerene nanomaterials inhibit the allergic response. *J Immunol* 2007;179:665–72.
- [111] Chan VS. Nanomedicine: an unresolved regulatory issue. *Regul Toxicol Pharmacol* 2006;46:218–24.
- [112] Takizawa H. Diesel exhaust particles and their effect on induced cytokine expression in human bronchial epithelial cells. *Curr Opin Allergy Clin Immunol* 2004;4:355–9.
- [113] Lofroth G. Environmental tobacco smoke: overview of chemical composition and genotoxic components. *Mutat Res* 1989;222:73–80.