CHAPTER 17

EFFECTS OF NANOPARTICLES ON THE PULMONARY VASCULATURE

Laurel E. Plummer
Kent E. Pinkerton
Amy K. Madl
Dennis W. Wilson

INTRODUCTION

Nanoparticles (NPs) are ubiquitous in the atmosphere and arise from both natural and anthropogenic sources. Increased concern over exposure to NPs has developed due to epidemiological findings that support the hypothesis that inhalation of these materials is associated with the induction and exacerbation of cardiovascular disease (CVD) and related CVD risk factors. These associations have spurred the scientific community to try to understand the mechanisms of the cardiovascular responses to inhaled ultrafine particles and NPs. As a result, several plausible mechanistic hypotheses for the development of cardiovascular effects within the pulmonary vasculature have been proposed. Involvement of the pulmonary vascular bed in particulate-induced health effects is plausible due to its location, structure, function, and cellular makeup and its involvement in the inflammatory and oxidative response to inhaled agents. Inhalation of NPs may induce or exacerbate preexisting cardiovascular conditions through inflammatory or oxidative stress pathways arising from the pulmonary epithelium or vascular endothelium and result in cellular damage and subsequent loss of function. Epidemiological studies and studies using human, animal, and cellular systems have been done to elucidate the key physical, chemical, spatial and temporal characteristics of NP-induced health effects and are summarized in a recent review (Mills et al., 2007). This chapter provides an anatomical and physiologic framework for further discussion of mechanisms of pulmonary vascular impacts of NPs in the context of major cellular players within the pulmonary vasculature.

Cardiovascular Effects of Inhaled Ultrafine and Nanosized Particles,
Edited by Flemming R. Cassee, Nicholas L. Mills, and David Newby

THE PULMONARY VASCULATURE

Anatomy and Structure

The circulatory anatomy of the respiratory tract provides multiple sites for close interaction between inhaled particles, epithelial and mesenchymal cells that interact with inflammatory cells, and microvascular conduits with reduced movement of blood elements due to hemodynamic forces. There are four primary regions for interaction between the external and internal environments: (1) bronchovascular bundles and submucosal capillary beds, (2) alveolar capillaries, (3) interstitial fluid, and (4) the nasal cavity. The bronchovascular bundle and associated submucosal capillary beds offer two-way trafficking for epithelial-derived signals to elicit both recruitment of inflammatory cells and activation of the microvascular endothelium and circulating elements of the blood. The alveolar capillary is the primary site for inflammatory cell activation and sequestration, which differs from most tissues where the postcapillary venule is the principal site of adhesion molecule expression and inflammatory cell transmigration (Krieglstein and Granger, 2001; David et al., 2009). Interstitial fluid may contain secreted paracrine signaling molecules from interstitial macrophages capable of interacting with alveolar capillaries and is present in distinct fluid and circulatory compartments formed by the bronchovascular bundle and junctions of the pulmonary parenchyma with the pleura and by the parenchyma and interlobular septa (Krieglstein and Granger, 2001; Semaeva et al., 2008). Lastly, the vascular bed of the nasal cavity is closely associated with the external environment and contains a metabolically active epithelium capable of transmitting paracrine signals to microvascular structures. Inhaled NPs may interact with several metabolically active cell types present within each of these sites resulting in procoagulant activities and systemic inflammation in the context of a reactive vasculature.

The complex and tightly regulated vasculature of the respiratory tract interacts with the systemic circulation. The right ventricular flow of oxygen-poor blood to the lungs through pulmonary arteries is augmented by that from the bronchial artery, which arises from the aorta and carries oxygenated blood. The extent to which the bronchial artery perfuses the bronchovascular bundles and submucosa of the airways varies markedly between species (McLaughlin, 1983). The bronchial artery eventually anastomoses with the pulmonary circulation with subsequent return to the heart through the pulmonary veins. The extent of bronchial artery-derived vasculature varies between species. Bronchial arteries end at the pulmonary hilus in the mouse but support a distinct submucosal vasculature that extends to the respiratory bronchiole in man. Pulmonary arteries and arterioles are specialized to function in a low resistance circuit while maintaining high flexibility in adjusting regional flow to balance with variable alveolar oxygenation. Compared with systemic arterioles, pulmonary arterioles are shorter, have thinner walls, contain less smooth muscle and elastin, and have lower average systolic and diastolic pressures. Relative perfusion of the visceral pleura by the pulmonary artery or bronchial supply varies by species with a venous return that appears to be through the pulmonary veins but also has a significant component of lymphatic flow (Albertine et al., 1982).

Function and Regulation

The pulmonary vasculature facilitates the close interaction between the external and internal environments, thus making it both a target and a gateway for particulate exposure. The main function of the pulmonary vasculature is to ensure the oxygenation of blood. Regulatory mechanisms facilitate the maintenance of blood flow to distribute oxygen-rich blood to the body via the systemic circulation, allowing for efficient ventilation and perfusion. Under stable conditions, blood moves from the right ventricle across the endothelial lining of the pulmonary vasculature before returning to the heart for systemic distribution. Blood flowing along intact healthy endothelium is not likely to clot or support platelet activation and thrombus formation within the lumen of the vasculature (Ryan, 1987). A healthy endothelium is maintained through the involvement of vascular endothelium, alveolar epithelial cells (AECs) I and II, leukocytes, and circulating platelets and erythrocytes that orchestrate the initiation, resolution, and repair of cellular insult.

Direct interaction of pulmonary cells with particles or downstream inflammatory mediators leads to the involvement of additional regulatory cascades that are balanced to activate and inhibit thrombogenic or coagulation events. Endothelial cell activation is signaled by the synthesis and secretion of proinflammatory mediators such as proinflammatory cytokines to respond to the insult as seen following exposure to bacterial endotoxin (Stoll et al., 2004). Expression of vascular adhesion molecules (VCAMs) allows for movement of inflammatory cells from the circulation through the interstitium and into the airway lumen. Thromboplastin and fibrinolytic factors, such as tissue plasminogen activators (tPAs) and urokinases, function to initiate blood coagulation and counter coagulation events, respectively, to maintain hemostasis or support clotting events (Ryan, 1987). Smooth muscle cells function to regulate vascular pressure and modify output based on the state of the vascular bed. The activity of crucial vasoregulators may be altered by oxidative or proinflammatory signaling.

Cell function and regulation is mediated through autocrine and paracrine signaling of biochemical mediators derived from activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) redox-sensitive and proinflammatory pathway. This pathway includes a protein complex controlling DNA transcription to respond to various stimuli, such as diesel exhaust and ambient particles (Bonvallot et al., 2001; Churg et al., 2005). Healthy and stable vascular conditions support the initiation and efficient resolution of vascular injury. However, cellular damage due to disease impedes appropriate biochemical responses and can exacerbate existing cardiovascular problems.

Pulmonary Macrovascular Function The pulmonary macrovasculature includes the larger vessels with an internal diameter of more than 100 μm. Pulmonary vascular tone is regulated by autonomic innervation, local paracine responses to pressure, and shear stress and alveolar oxygen tension. Pulmonary arterioles and, to a lesser extent, pulmonary veins are innervated by sympathetic nerve fibers that stimulate vasoconstriction (Barnes and Liu, 1995). These regulate physiological responses to stress and exercise. Local modulation of vascular tone is largely an

interaction between endothelial and smooth muscle cells through mediators that include nitric oxide (NO), endothelin, and prostanoids, which are in turn modulated by circulating vasoactive substances and products of inflammatory or coagulation cascades. Similar to the systemic circulation, pulmonary artery endothelium is sensitive to changes in flow. Cardiac defects leading to high pulmonary artery flow drive vascular remodeling that increases resistance. *In vitro*, pulmonary endothelium is polarized under conditions of flow and, under static conditions, depolarization is associated with activation of ATP-dependent K+ channels that lead to increases in endothelial nitric oxide synthase (eNOS) (Chatterjee et al., 2003). This suggests that, *in vivo*, decreased flow stimulates NO secretion to cause vasodilation. Pulmonary vessels are also responsive to mechanical stretch, both in response to the cardiac cycle and as a consequence of ventilatory movements. These responses are likely mediated by the transient receptor potential ion channel family through Ca++ mediated messaging (Yin and Kuebler, 2009).

In addition to providing a low resistance and thus slow flowing circuit to maximize oxygen exchange, the pulmonary vasculature directs alveolar perfusion to match ventilation. Regional alveolar hypoxia induces arterial constriction through mechanisms that appear to involve endothelial cell sensing of oxygen tension through mitochondria. Pulmonary endothelial cells have an increased sensitivity for hypoxia that rivals that of carotid body receptors (Mark Evans and Ward, 2009). Local hypoxia thus elicits endothelial cell-derived vasoconstrictive factors that reduce blood flow to underventilated alveoli and prevent shunting of unoxygenated blood to the systemic circulation.

The basis for the development of pulmonary hypertension is the disruption of regulation systems of pulmonary vascular resistance. While vasoconstrictive pulmonary hypertension can be a serious consequence of acute lung syndromes like adult respiratory distress syndrome (ARDS), these syndromes are somewhat amenable to pharmacological treatment. Prolonged hypertension is accompanied by the remodeling of the vascular wall that is much more refractory to treatment. The small pulmonary arterioles are the principal site of increased resistance in pulmonary hypertension. Initial remodeling by hypertrophy and hyperplasia of smooth muscle in the arteriolar wall evolves toward a myofibroblastic smooth muscle phenotype that synthesizes elastin and collagen. The increase in extracellular matrix causes loss of vascular wall compliance that eventually drives right ventricular failure. Diverse causes and mechanisms contribute to pulmonary arterial remodeling in several syndromes. Secondary pulmonary hypertension may be related to congenital heart defects (shear stress), chronic hypoxia, or concurrent pulmonary diseases, such as pulmonary fibrosis (mechanotransduction). Primary pulmonary hypertension occurs in the absence of other contributing cofactors. Genetic analysis of familial primary pulmonary hypertension implicates receptors in the transforming growth factor-beta (TGF-β) family, specifically BMPRII, in this disease. Other associations with pulmonary hypertension include HIV, scleroderma, and anorexigenic drugs. Whether similar genetic cofactors influence the incidence of pulmonary hypertension in the later syndromes is an area of active investigation.

In contrast to the evidence that environmentally derived NPs influence systemic vascular disease (Dockery, 2001), there is little epidemiological or experimen-

tal evidence that inhalation of NPs alters pulmonary vascular resistance. The strongest indications come from studies of environmental or sidestream tobacco smoke (ETS), which consists of both vapor and solid-phase particulates and gases. Studies with ETS-exposed animals suggest that pulmonary vascular remodeling may occur (Nadziejko et al., 2007). ETS-induced angiogenesis and leukocyte trafficking in the lungs of mice is indicated by increased rolling and adhesion of neutrophils in lung microvessels and increased vascular density as compared with filtered air-exposed mice (Rao et al., 2009). Epidemiological evidence from ETS-exposed airline workers correlates only with systemic hypertension (Ren et al., 2008).

There is some evidence that pulmonary hypertension may be an important cofactor for sensitivity to NP-induced health effects. Systemic hypertension was found to be a cofactor in hospital admissions during elevated particulate matter (PM) episodes, but more data are needed to know whether pulmonary hypertension is a cofactor (Peel et al., 2007). Most data come from the evaluation of monocrotaline (MCT)-treated rats, which rapidly develop progressive pulmonary arterial remodeling and right ventricular hypertrophy (for a review, see Wilson et al., 1989). Death from pulmonary hypertension is often a consequence of right ventricular failure. Initial work with particle inhalation using the MCT model showed that right ventricular failure was associated with increased cardiac arrhythmias in fly ash-instilled rats (Watkinson et al., 1998). Further work demonstrated that intratracheal instillations of fly ash resulted in a high rate of mortality in MCT-treated rats. Inhalation exposure resulted in augmented markers of pulmonary inflammation in MCT-treated rats but did not result in mortality (Kodavanti et al., 1999). Rats compromised by MCT treatment show exacerbated ventilatory responses with delayed recovery after fly ash inhalation (Gardner et al., 2004). In addition to the cardiopulmonary effects of MCT treatment, it appears that the MCT rat model also interferes with particle clearance, leading to increased retention times and greater particle interactions with biological systems (Madl et al., 1998).

Respiratory Microvascular Function Three microvascular regions of the respiratory tract have potential for close interaction between the external environment, mucosal epithelium, and capillary endothelium: (1) the nasal cavity, (2) the submucosal capillaries of the bronchovascular bundles and bronchial submucosa, and (3) the alveolar capillaries. The nasal cavity is a highly vascularized structure with extensive surface area adjacent to the external environment. In humans, this represents a mucosal surface area of 170 cm^2 and is designed for warming and humidifying air through thin capillaries (Hussain, 1989). The connection of the external environment and thin capillary beds of the nasal mucosa facilitates bidirectional movement of fluids to humidify air and uptake of aerosols into the bloodstream (Mygind and Vesterhauge, 1978). A second microvascular space consists of the submucosal capillaries of the bronchovascular bundles and bronchial submucosa. This circulation, derived from the bronchial artery in most species, interfaces with signals emanating from bronchial epithelial cells to recruit circulating inflammatory cells that undergo transepithelial migration in response to inhaled irritants and infectious agents. An associated consequence of inflammatory stimuli is the microvascular leak that accentuates submucosal edema. A third microvascular space contains

the largest vascular surface area; approximated in virtually all mammalian species to be an area 75–95% of the total measured alveolar surface area is the network of alveolar capillaries (Pinkerton et al., 1992). The alveolar capillary bed is an integral component of the septal structures forming the walls of each alveolus. Interconnected capillaries are supported by interstitial connective tissue that interweaves to polarize endothelial cells such that the nucleus is subtended by connective tissue and located on the abluminal side of the endothelial cytoplasm's interface with alveolar epithelium. Capillary endothelial cells synthesize an extracellular basement membrane that fuses with the basement membrane formed by type I and type II pneumocytes (AECs I and II) to ensure a thin air-to-blood tissue barrier for gas exchange. AEC I is composed of squamous epithelial cells that cover up 95% of the entire alveolar surface. These cells rarely divide and are replaced by differentiating AEC II when damaged (West et al., 1998). AEC II is cubodial and contains numerous intracytoplasmic lamellar inclusions, which, when actively secreted from the cell, form the surfactant lining of the alveoli that reduces surface tension (West et al., 1998).

The alveolar microvasculature is unique in that it is relatively impermeant and has a high capacity for fluid resorption by vesicular transport (Effros and Parker, 2009). The extracellular matrix in the alveolar wall is also specialized to limit fluid accumulation. High concentrations of heparan sulfate proteogylcans bind to proteins to regulate angiogenesis and coagulability, limit fluid permeability through the basal lamina, and provide elastic stretch that increases interstitial hydrostatic pressure when fluid does accumulate (Miserocchi, 2007). The alveolar capillary bed is also a significant reservoir of marginated neutrophils, thus differing from other tissues where neutrophil margination occurs in postcapillary venules (Schwab et al., 2003). It is evident that the alveolar microvasculature is specialized to limit the accumulation of edema fluid yet uniquely susceptible to inflammatory-induced disruptions of the fluid barrier.

Regulation of lung interstitial fluid flux is a multifactorial process involving active transport by alveolar epithelium and endothelium, hydrostatic pressure differentials based both on extracellular matrix and the surface tension reducing action of surfactant, and a highly efficient lymphatic sump. The latter is aided by the construction of the bronchovascular bundles, which are relatively isolated fluid spaces, and tether vessels to their walls, thus allowing adjacent alveolar airspaces to hold vessels open despite changes in intrapulmonary pressures (Miserocchi, 2007). The consequence of the unique and dynamic interstitial fluid compartments in the lungs is that this creates a microenvironment where the admixture of plasma-derived activities and regulatory cytokines secreted by pulmonary epithelial cells can interface with multiple capillary beds, resulting in potential endothelial cell activation.

IMPLICATIONS OF PULMONARY VASCULAR INJURY

Exposure to elevated levels of NPs has been shown to induce acute changes such as endothelial dysfunction, platelet activation, and alterations to coagulation and fibrinolytic cascades that may contribute to chronic conditions such as atherosclerosis and pulmonary hypertension. For example, acute damage to the capillary

endothelium and AEC I causes disruption of the tight junctions and edema or the presence of a protein-rich fluid in the alveolar space, which, if not removed, leads to further epithelial injury and cell death. Pulmonary edema develops in two stages, interstitial edema and alveolar edema, with the latter preventing ventilation and leading to hypoxemia. Increased leakage of the pulmonary tight junctions also facilitates the movement of inflammatory mediators between the systemic circulation and pulmonary epithelium.

Clinical Studies

Physiological research with both human subjects and animal models provides increasing evidence for cardiovascular effects of ultrafine particles and NPs. Physiological studies with human subjects suggest potential pulmonary and systemic inflammatory and procoagulant mechanisms, linking air pollution to observed pathogenesis of atherosclerosis, myocardial infarction, and pulmonary hypertension. While many measured physiological end points are systemic, it is a valid hypothesis that inflammation initiated in the pulmonary vasculature may have subsequent systemic effects. Pathophysiological changes involving several potential mediators of the above health effects—inflammation, coagulation, and cardiac rhythm—were observed in a study of healthy men exposed to ambient particles (Riediker et al., 2004). Cardiac arrhythmias, or abnormal electrical activity of the heart, were increased in elderly subjects exposed to ambient particles, and effects were seen with a lag time of 1–2 days (Peters et al., 2000). Ultrafine ambient particles induced inflammatory and prothrombotic responses in healthy human volunteers (Samet et al., 2009). Healthy human volunteers exposed to diesel exhaust particles (DEPs) demonstrated increased thrombus formation and increased neutrophil-platelet and monocyte-platelet aggregates compared with filtered air-exposed control subjects (Lucking et al., 2008). In a separate study, diesel exhaust-derived particle exposure induced mild systemic inflammation and resulted in impaired endothelium-dependent vasodilation, a possible risk factor for pulmonary hypertension (Tornqvist et al., 2007). Mills and colleagues demonstrated alterations in the regulation of vascular tone and endogenous fibrinolysis in the absence of differences in resting blood flow or systemic inflammatory markers in humans (Mills et al., 2005a,b). Controlled human studies demonstrated alterations in blood leukocyte distribution and expression of adhesion molecules, suggesting an influence of systemic inflammation on tissue inflammation (Frampton et al., 2006). Similarly, human subjects with occupational or environmental PM exposure had altered or significantly elevated levels of adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1), von Willebrand factor (vWF) antigen, plasminogen activator inhibitor-1 (PAI-1), and high sensitivity C-reactive protein (hs-CRP), suggesting stimulation of inflammation and coagulability associated with PM exposure (Rueckerl et al., 2006; Bigert et al., 2008). A recent study suggests a role for urban PM, but not the manufactured particles of carbon black and titanium dioxide NPs, in endothelial dysfunction through the impairment of endothelial NO-dependent relaxation in intrapulmonary arteries, possibly contributing to urban PM-induced cardiovascular dysfunction (Courtois et al., 2008).

Physiological consequences of cellular and biochemical alterations induced or exacerbated by particulate exposure include increased atherosclerotic plaque instability with the potential for plaque rupture or thrombosis resulting in myocardial infarction or stroke (Vermylen et al., 2005). Potential side effects from inflammation, such as endothelial dysfunction and platelet activation, may lead to the development of thrombi. Manufactured carbon nanotubes (CNTs) and ambient ultrafine particles have been found to induce platelet activation in humans and animal models, which may play a role in the abnormal response to NPs in general (Nemmar, 2003; Nemmar et al., 2003, 2004, 2007, 2009; Delfino et al., 2008, 2009; Semberova et al., 2008). Clinical results like these are not always reproducible and are most likely dependent on additional factors, such as chemical composition of the NPs (Riediker et al., 2004; Brauner et al., 2008).

Animal Studies

Animal models of susceptibility to cardiovascular and endothelial dysfunction have contributed to the understanding of the effects of ambient air pollution on the role of inflammatory mediators derived from epithelial, endothelial, and inflammatory cells. Systemic measures may indicate a role for these cell types in the initiation of inflammation, endothelial activation and dysfunction, and edema within the respiratory tract. The models include aged or spontaneously hypertensive animals and those susceptible to the development of atherosclerotic lesions, such as apolipoprotein E-deficient mice ($ApoE^{-/-}$ mice) and low-density lipoprotein (LDL) receptor knockout mice. Long-term exposure to low concentrations of $PM_{2.5}$, particles with a mean mass aerodynamic diameter less than 2.5 μm including ultrafine particles, induces vascular inflammation and potentiated atherosclerosis in $ApoE^{-/-}$ mice (Sun et al., 2005a,b). Acute exposures to concentrated ambient particles (CAPs) led to larger early atherosclerotic lesions in ApoE-deficient mice exposed near a freeway in Los Angeles, CA (Araujo et al., 2008). This phenomenon has been observed in other animal models of atherosclerosis exposed to environmental NPs (Suwa et al., 2002; Sun et al., 2005a,b). Two recent studies examine endothelial responses in healthy and hypercholestrolemic rabbits (Tamagawa et al., 2008; Yatera et al., 2008). Tamagawa and colleagues demonstrated that repeated exposure to PM-induced lung and systemic inflammation as well as endothelial dysfunction (Tamagawa et al., 2008). Yatera and colleagues concluded that exposure to ambient air pollution particles promoted the recruitment of circulating monocytes into atherosclerotic plaques, and they further speculated that this is a critically important step in particle-induced progression of atherosclerosis (Yatera et al., 2008). Particle instillation altered hemostasis in exposed mice, suggesting that particle exposure supports clot formation and inhibits clot resolution in susceptible subjects (Cozzi et al., 2007). Healthy and compromised aged rats exposed to freshly generated diesel exhaust demonstrated significant particle-associated increases in plasma endothelin-2 (ET-2) suggestive of vascular endothelial cell activation (Elder et al., 2004). Controlled exposure to ambient particles induced an elevation in arterial blood pressure in dogs and similar changes in indices of cardiovascular effects in spontaneously hypertensive rats (Wichers et al., 2004; Bartoli et al., 2009). Carbon black NPs induced pulmonary

edema due to loss of cellular barrier function (Inoue et al., 2006). Pulmonary edema as a marker of pulmonary toxicity of ultrafine particles and NPs was observed in mice exposed to CNTs and carbon black NPs in the lungs of rats following systemic administration of DEPs (Nemmar et al., 2007; Tong et al., 2009).

MECHANISMS OF PULMONARY VASCULAR INJURY

Current knowledge regarding interactions of ultrafine particles and NPs with cells and biological systems is derived from studies of the cellular response to microorganisms, such as bacteria and viruses. However, inorganic ambient and engineered NPs often persist much longer than the biologically active microorganisms (Buzea et al., 2007). Investigations of various NP types with target cells and biological systems have suggested mechanisms for the development of CVD through the interface of oxidative and inflammatory responses and mediators and consequences of their effects in the circulation.

Activation of oxidative and inflammatory pathways is a common theme in NP-induced toxicity and experimental research (Buzea et al., 2007). However, precise cellular targets leading to subsequent adverse cardiovascular health effects are still under investigation. Several hypotheses regarding cell–particle interactions are summarized in a recent review and include direct and indirect initiation of inflammation and oxidative stress (Polichetti et al., 2009). NPs may induce mild pulmonary inflammation and oxidative stress triggering a subsequent alveolar and systemic inflammatory response, ultimately leading to tissue damage (Seaton et al., 1995). NPs may also directly translocate into the circulation after deposition in the distal lung and interact directly with endothelial cells and other extrapulmonary targets resulting in induction of inflammatory and oxidative stress cascades there (Kreyling et al., 2002, 2009; Oberdorster et al., 2002, 2004). These hypotheses suggest the involvement of a number of cellular targets (bronchial epithelial cells, alveolar macrophages (AMs), AECs, and vascular endothelial cells) in the orchestration of the oxidative and inflammatory response to inhaled ultrafine particles and NPs.

Inflammation and Oxidative Stress in the Pulmonary Vasculature

Pulmonary inflammation contains all the classic aspects of acute responses to injury, including vasodilation, microvascular permeability, activation of proinflammatory and procoagulant enzymatic cascades, activation and chemotaxis of inflammatory cells, and secretion of hematogenous signals driving systemic responses and myeloid activation. Traditional concepts of pulmonary inflammation relative to inhalation toxicology focus on mediators derived from bronchial epithelium. The role of the alveolar surface in inflammation is largely considered in relation to diffuse alveolar injury, pulmonary edema, and intracapillary inflammation associated with acute respiratory distress (ARDS-like syndromes). Evidence for alveolar deposition of ultrafine particles and NPs at the alveolar surface raises the possibility that these particles induce signaling in the alveolar wall and may contribute to vascular and

systemic responses to particle inhalation. The initiation of inflammation within the pulmonary or vascular lumen has the potential for cellular damage that may drive downstream systemic cardiovascular effects observed epidemiologically and experimentally. Extracellular signaling in inflammation is commonly measured as an indicator of the involvement of pulmonary epithelial and endothelial cells, macrophages, and neutrophils. Signaling molecules secreted by these cellular players include cytokine and chemokine proteins, leukotrienes, and prostaglandins. Intracellular signaling controls the production and secretion of these extracellular signaling molecules that then act by both autocrine and paracrine mechanisms to propagate an inflammatory event.

Microvascular Metabolism and Inflammation

The pulmonary microvasculature is ideally situated to perform metabolic homeostasis for circulating elements of the blood because it receives the entire cardiovascular output over a large surface area with flow that is regulated to be slow. The capillary endothelium of the lungs has metabolic and inflammatory pathways that are distinct from that of large vessels (Stevens et al., 2008). A large body of work has characterized a plethora of endothelial activities, many of which are particularly prominent in the pulmonary microvasculature. These can be categorized as metabolism of circulating biologically active molecules, local signaling that directs vascular contractility, or proliferative responses and interaction with inflammatory and coagulation cascades.

In addition to inducing a proinflammatory environment in the pulmonary microvasculature through lipopolysaccharide (LPS)-mediated mechanisms, pulmonary epithelial or AM-derived cytokines, specifically tumor necrosis factor-alpha (TNF-α) and interleukin-8 (IL-8), have the capacity to elicit endothelial activation. As described below, inhalation of NPs can elicit epithelial and macrophage responses that include secretion of TNF-α and IL-8. This, in combination with the likely greater alveolar deposition of NPs relative to larger particles, makes the alveolar microvasculature an ideal microenvironment for the transmission of NP-induced pulmonary responses to the systemic circulation.

Effects of NP Chemical Composition on Vascular Injury

Toxicological comparisons between difference sizes, morphology, and compositions of ultrafine particles and NPs have been conducted to understand how physical and chemical characteristics influence biological and cellular response within the pulmonary cells and vasculature (Clift et al., 2008; Kreyling et al., 2009). All particles less than 100 nm in one dimension do not behave equally, and their retention and clearance patterns differ significantly from their larger counterparts. NP translocation and effects occurring distant from deposition sites have received considerable attention, primarily because NPs have a potentially high efficiency for deposition, target both the upper and lower regions of the respiratory tract and are retained in the lungs for a longer period of time, induce more oxidative stress, and cause greater inflammatory effects than their fine-sized equivalents (Ferin et al., 1992; Ferin, 1994; Oberdorster et al., 2002; Semmler-Behnke et al., 2007). Retention times for radio-

labeled 100-nm carbon NPs appear to be over 95% of deposited particles with a greater percent of deposition for current smokers or chronic obstructive pulmonary disease (COPD) patients compared with healthy individuals (Moller et al., 2008). Further, the inverse relationship between size and surface area contributes to a greater surface for adsorption of chemicals, which is thought to influence biological responses in the pulmonary vasculature that may be exacerbated by the increased retention times of these materials.

Particle size, morphology, and chemical composition are believed to be important factors in the alteration of pulmonary microvascular endothelial function. Mechanistic studies have been performed using combustion-derived, lab-generated, and ambient NPs as a component of $PM_{2.5}$ to investigate the influence of composition and size on particle uptake (Clift et al., 2008), exogenous and endogenous redox activity (Becker et al., 1996; Brown et al., 2004; Cho et al., 2005), and cytotoxicity and proinflammatory cytokine responses (Becker et al., 1996, 1999, 2003, 2005; Veranth et al., 2006; van den Bogaard et al., 2009). Specifically, exposure of human aortic endothelial cells (HAECs) to iron oxide, yttrium oxide, and zinc oxide resulted in composition-dependent inflammatory responses that showed consistent surface interactions and uptake of all NPs (Gojova et al., 2007).

NPs are derived from combustion processes and have the potential for aggregation forming accumulation mode particles with very unique chemical characteristics (Churg et al., 2005). The chemical composition of ultrafine particles and NPs reflects their source as transition metals, and polycyclic aromatic hydrocarbons (PAHs) are the primary components of these ambient particles. Engineered NPs are also high in carbon and metals due to the unique properties of these materials that facilitate their use in emerging electronic and consumer product technology. Research has shown relationships between the levels of carbonaceous and metallic components and induction of inflammation and oxidative stress in biological systems.

In general, particulates are an exogenous source of reactive oxygen species (ROS) because they possess an innate capacity to catalyze the generation of ROS, which is frequently used as a measure of toxicity. NPs, produced through combustion processes, contain high amounts of transition metal components and other redox cycling compounds, such as organic carbon species. The ability of ultrafine particles ($PM_{0.1}$) to generate more free radicals than coarse (PM_{10}) and fine particles ($PM_{2.5}$), as measured by induction of heme oxygenase and depletion of intracellular glutathione, may be due to ultrafine particles having a large surface area for adsorption of ROS-generating components (Cho et al., 2005). Inherent ROS generation is commonly associated with transition metals, such as iron (Fe), copper (Cu), nickel (Ni), cobalt (Co), and vanadium (V). These metals are associated with anthropomorphic sources, with Fe having an order of magnitude greater concentration in most samples from polluted regions (Deguillaume et al., 2005). Transition metals such as Fe catalyze the production of hydroxyl radical from hydrogen peroxide via Fenton-like reactions that are enhanced in the presence of physiological concentrations of ascorbate (Vidrio et al., 2008).

In addition to transition metals, environmental PM contains PAHs and quinones that undergo redox cycling to generate ROS. In the burning of hydrocarbons, radicals formed early in combustion interact, forming PAHs, including carcinogens,

from less complex structures. PAHs will aggregate into NPs, which can extend into branched-chain structures observed as black smoke or soot (Rouse et al., 2008). Quinones are derived from PAH components and likely include such compounds as 1,4-naphthoquinone, 5,12-naphthacenequinone, bez[*a*]anthracene-7,12-dione, and anthracene-9,10-dione. Quinones undergo cyclic reduction reactions with oxygen followed by oxidative coupling with either nicotinamide adenine dinucleotide phosphate (NADPH) or iron (Squadrito et al., 2001).

Endogenous sources of ROS are generated by many cellular and enzymatic players. A primary source of ROS is the generation of a wide variety of oxidants through phagocytic respiratory bursts of macrophages and neutrophils. Much of the understanding of cellular-based synthesis of ROS was developed through investigation of antimicrobial systems used by leukocytes in which bacterial killing is facilitated by the generation of a variety of highly effective bactericidal agents, including superoxide anion, hydrogen peroxide, and halo-oxygen species such as HOCl. The respiratory burst is a metabolic event in which cells manufacture large quantities of highly reactive oxidants in response to a stimulus and is a cyanide-insensitive rapid utilization of oxygen and glucose. Leukocytes, such as neutrophils and macrophages, produce ROS during phagocytosis or stimulation with a wide variety of agents through activation of NADPH-reduced oxidase (NOX) (Forman and Torres, 2002). The capacity for this rapid response is regulated by protein phosphorylation of key subunit, $p47^{phox}$, a 45-kDa oxidase component that is extensively phosphorylated when the oxidase is activated. This subunit moves to the phagosomal membrane where, as it organizes with the remaining components of the active enzyme, it generates electrons to reduce molecular oxygen (Raad et al., 2009). The organized oxidase complex acts through a catalytic subunit ($gp91^{phox}$, also known as NOX2) to bind NADPH, extract electrons, and transport them across the phagosomal membrane to react with oxygen through an iron–heme complex, thus secreting superoxide into the lumen (Lambeth et al., 2007). Differentiated macrophages (such as AMs) also generate ROS using NOX2-based metabolism but lack the myeloperoxidase present in neutrophils, which is necessary for HOCl generation. Macrophages also generate nitrogen-based radicals through nitric oxide synthase (NOS) and its interaction with active oxygen species. Nonphagocytic cells use similar reactive oxygen-generating enzyme complexes for both innate defense and intracellular signaling (Boueiz and Hassoun, 2009).

CELL-SPECIFIC RESPONSES

Initial contact with inhaled pathogens and toxins occurs in the epithelium. It is increasingly clear that receptor-mediated stimulation of epithelial proinflammatory molecule secretion is the initiating step of many pulmonary inflammatory processes. Of the resident pulmonary cells, microvascular endothelium, airway and alveolar leukocytes, pulmonary epithelium, and airway dendritic cells are the most significant regulators of inflammatory responses (Fig. 17.1). Macrophages and dendritic cells often act indirectly through the secretion of cytokines, while endothelial cells have more direct interaction with the circulation through both secretion and cell surface expression of proinflammatory and adhesion molecules. Oxidants have the potential

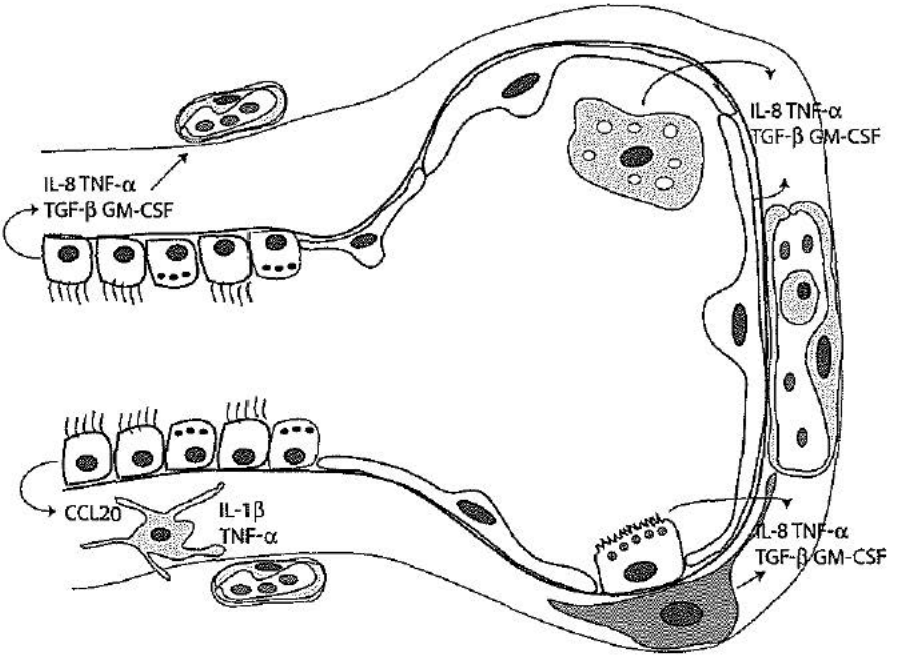

Figure 17.1 Diagram of an alveolar sac in the distal lung demonstrating airway epithelium, alveolar epithelium, dendritic cells, alveolar macrophages, and endothelial cells and associated mediators of inflammation derived from these cells.

to interact with the wide variety of cell types in the lungs and are also produced naturally by a subset of cells involved in phagocytosis of foreign pathogens.

Due to their small size, NPs evading phagocytic clearance may diffuse into the pulmonary circulation where they have direct interaction with interstitial and endothelial cells and may induce or respond to inflammation and oxidative stress. The idea that NPs access the circulation following inhalation and elicit direct effects in the circulation and secondarily target organs is controversial but has been illustrated in several animal model studies (Kreyling et al., 2002, 2009; Oberdorster et al., 2002; Nemmar et al., 2001, 2002; Nemmar, 2003). An alternative mechanism connecting pulmonary responses to NP inhalation to systemic responses may be through induction of inflammation at the juncture of the pulmonary and systemic circulation. Mechanisms of NP-induced activation of pulmonary target cells that could contribute to this process are described below.

Bronchial Epithelial Cells

The nature of NPs governs particle deposition throughout the respiratory tract, from the proximal to the distal lung. The mechanisms of receptor activation leading to cytokine production in epithelial cells following particle exposure remain incompletely characterized. Two principal hypotheses are activation of Toll-like receptors

(TLR) responses and ROS-mediated signaling. Much is known about airway epithelial cell responses to infection (Message and Johnston, 2004). Bronchial epithelium participates in innate and immune host defense responses with patterns specific to the underlying pathogens. In the case of viral infection, the principal response is secretion of interferon with immune defenses focusing on stimulation of dendritic cells, antigen presentation, and lymphocyte recruitment. A broader assortment of innate defense molecules characterizes the response to bacterial infection. These include secretion of antibacterial peptides, such as defensins, collectins, and cathlicidins, and antimicrobial actions of surfactant and Clara cell secretory proteins (Grubor et al., 2006a,b).

TLRs are key components for the recognition of infectious agents. Airway epithelial cells express TLRs 2–6. TLR-2, when dimerized with TLR-6, recognizes bacterial products, such as lipoprotein and peptidoglycan as well as some components of protozoa and viral envelope proteins. TLR-3 recognizes double-stranded RNA and thus responds largely to viral infection, while TLR-4 recognizes LPS associated with Gram bacteria. TLR-5 responds to bacterial flagellin and is therefore important in specific bacterial infections, such as those involving pseudomonas. TLR signaling acts through NF-κB and interferon regulatory pathways to upregulate cytokines, such as TNF-α, IL-6, and IL-12 or type I interferon in a pathogen-specific context (Schleimer et al., 2007).

Viral infection also acts through alternate epithelial cell receptors. In the case of rhinovirus, initial infection of bronchial epithelial cells occurs by attachment to ICAM-1 with subsequent augmented ICAM-1 expression resulting in progression of infection. Rhinoviruses are also reported to activate an epithelial cell LDL receptor. Epithelial cells respond by secreting a host of proinflammatory molecules including IL-1α, IL-1β, IL-6, IL-11, IL-16, TNF-α, granulocyte macrophage colony stimulating factor (GM-CSF), IL-8, Gro-β, epithelial neutrophil activating protein (ENA)-78, regulated upon activation, normal t-cell expressed and secreted (RANTES), eotaxin 1 and 2, and macrophage inflammatory protein (MIP)-1α (Message and Johnston, 2004).

TLR involvement is thought to reflect relative contamination of PM with bacterial products. One study comparing ambient source PM with diesel exhaust and silica demonstrated that ambient PM stimulated TLR-2 responses in bronchial epithelial cells, while AMs responded through TLR-4 signaling. In contrast, diesel particles did not stimulate cytokine release and suppressed TLR expression (Becker et al., 2005). Autocrine enhancement of TLR receptors being involved in IL-6 production is evidenced by TLR-2 expression being upregulated by animal agricultural dust (Bailey et al., 2008). Further support for the role of bronchial epithelial cells in inflammation is the expression of lysophosphatidic acid (LPA) receptors. Stimulation of human bronchial epithelial cells (HBECs) with LPA causes upregulation of IL-8, cyclooxygenase (COX)-2, and IL-13 receptor α2 (IL-13Rα2) (Zhao and Natarajan, 2009). LPA is derived from phosphatidic acid through activation of phospholipase A1 or A2 and is a potent biological mediator of inflammation acting through NF-κB and AP-1 signaling pathways to stimulate inflammation.

Recognition that cells other than leukocytes express NOX-related enzymes has greatly expanded the perspective of ROS generation in response to injury.

Bronchial epithelial cells express members of the endogenous NOX family, Duox, which contains both superoxide and peroxidase catalytic sites. The expression of the two forms of Duox is differentially regulated such that t-helper cell (TH-1)-associated cytokines (IL-4, IL-13) induce Duox1, while TH-2 cytokines (IL-1α, IL-1β, and interferon [IFN]-γ) induce Duox2 (Lambeth et al., 2007). These complementary systems are thought to be important in secreting active oxygen species for antibacterial defense in bronchial lining fluid.

Experimentally, mice exposed to PAH-rich, petrochemical combustion-derived NPs have airway inflammation and induction of aryl hydrocarbon receptor (AhR)-associated oxidative stress and proinflammatory genes (Rouse et al., 2008). Similar oxidative stress and known markers of AhR ligand activity genes were observed when bronchial epithelial cells (BEAS-2B) were exposed to extracts of ambient PM collected from the United States–Mexico border (Lauer et al., 2009). Expression and activity of cytochrome P450 (CYP450) throughout the lungs provide insight into cytoprotective and antioxidant responses and metabolism of inflammatory mediators. Activation of CYP450 metabolism can contribute to ROS generation through uncoupling of the electron transport chain associated with the oxidative metabolism of xenobiotics catalyzed by CYP450. Some PAH moieties can occupy the active CYP site but are relatively inefficiently oxidized leading to the release of oxygen radicals generally used as electron donors by this pathway. A good example of this process has been demonstrated for polychlorinated biphenyls (PCBs) congeners and CYP1A1 (Green et al., 2008). Metabolism of the PAHs commonly associated with PM by CYP450-related enzymes is central to host responses to environmental and endogenous chemicals. The presence of PAHs in environmental PM is well documented, and activation of CYP isozymes is increasingly recognized as a stereotypic response. One of the most common pathways of reaction to PAH exposure is the activation of the AhR with subsequent upregulation of enzymes associated with the CYP1 family of P450 isozymes. Additionally, cell culture studies of BEAS-2B exposed to ambient PM demonstrated varying induction of neutrophil and dendritic chemokines CCL20, CXCL1/-3/-8/-10/-11, lymphotoxin (LT)-β, TNF-α and IL-6 by crystalline silica, ultrafine carbon black, $ZnCl_2$, $FeSO_4$, LPS, and 1-nitropyrene (Ovrevik et al., 2009).

AMs

Phagocytic leukocytes, such as AMs, neutrophils, and eosinophils, have long been considered the key receptor for inhaled particles. Leukocytes, specifically AMs, function in particle clearance pathways and drive the initiation of pulmonary inflammation beginning with cellular migration into the affected tissue, particle phagocytosis, and release of signaling molecules, including cytokines and other inflammatory mediators. NPs have been shown to inhibit phagocytosis in AMs as well as elicit inflammation and disrupt chemotaxis (Renwick et al., 2001, 2004). Much of what is known about AM function is derived from bacterial defenses, and there is much interest in the interaction between biologically derived components of environmentally derived PM and AM activation. Several studies implicate endotoxin (LPS) in inflammatory responses to both coarse and fine ambient PM (Becker et al., 2003,

2005). These responses seem relatively unique to environmentally derived PM as preparations of diesel exhaust and inorganic particles do not elicit similar responses (Becker et al., 2005). Alexis and colleagues demonstrated the effect of heat inactivation on the inhibition of AM responses (Alexis et al., 2006), and TLR-4 antagonists block responses to coarse environmental PM, suggesting the involvement of surface receptors in phagocytosis and subsequent release of proinflammatory signaling molecules (Becker et al., 2005; Alexis et al., 2006).

Conventional phagocytosis is mediated by surface ligands and receptors on microorganisms and the phagocytic cell. However, NP lacking these ligands can still reach the interior of the cell due to their small size. Additionally, a variety of particles have been shown to initially interact with macrophages through LDL scavenger receptors, and these interactions appear specific for charged particles and do not seem to be effective for carbonaceous particles, such as those derived from diesel exhaust (Kobzik et al., 1995). The LDL receptor pathway is also used for phagocytosis of unopsonized bacteria and does not initiate a respiratory burst. Furthermore, AM phagocytosis of particles does not occur through the SR I receptor but rather a related protein referred to as macrophage receptor with collagenous structure (MARCO) (Palecanda et al., 1999). While phagocytosis through the MARCO receptor is not associated with a macrophage respiratory burst, activation of cytokine release appears to be oxidant dependent.

The principal cytokine response following AM phagocytosis of particulates is the release of TNF-α and MIP-1 (Imrich et al., 1999). Antioxidants inhibit this response, and addition of exogenous or induced endogenous H_2O_2 enhances the cytokine response (Imrich et al., 2007). This suggests that endogenous ROS signaling may be a component of the AM response as evidenced by the activation of the mitogen-activated protein (MAP) kinase activated AP-1 transcription pathway (Singal and Finkelstein, 2005). Other AM-derived proinflammatory mediators include IL-1β (Huang et al., 2004), IL-6 (Becker et al., 2003), IL-8, and COX-2 (Becker et al., 2005). Interaction of AM with bronchial epithelial cells augments the expression of a variety of cytokines (Ishii et al., 2005). Other effects of particulates on AM function include production of markers of activation, such as CD11b and mCD14 HLA expression, increased phagocytosis, and inhibition of bacterial killing (Becker et al., 2003; Alexis et al., 2006; Mundandhara et al., 2006; Lundborg et al., 2007; Zhou and Kobzik, 2007).

Alveolar Epithelial Type I and II Cells

Alveolar type I epithelial cells (AEC I) are primarily structural in function, and knowledge of their participation in inflammatory events is limited due to the difficulty of maintaining them in culture (Chen et al., 2006). Recent *in vitro* studies have used an AEC I-like cell line transformed type I (TT1) to demonstrate secretion of proinflammatory cytokines IL-6 and IL-8 following LPS exposure (van den Bogaard et al., 2009). Borok and colleagues have conducted studies to understand additional physiological roles of type I cells and have localized sodium ion channels and pumps to the plasma membrane of AEC I, supporting AEC I's role in maintaining an appropriate layer of surfactant on cell surfaces (Borok et al., 2002a,b,c; Eaton et al., 2009). Chen and colleagues have observed upregulation of ApoE, a lipoprotein

involved in the transport of cholesterol and other lipids, and transferrin, a major plasma iron transport glycoprotein, by AEC I in hypoxic lungs, suggesting a protective response to oxidative stress (Chen et al., 2006).

The highly metabolic and easily cultured alveolar type II cells (AEC II) are capable of releasing a number of proinflammatory molecules in response to a variety of inflammatory stimuli (Koyama et al., 1998a,b). AEC II is also capable of internalizing both fine and ultrafine particles (Brandenberger et al., 2009). *In vitro* experiments to investigate the induction of inflammation have been conducted with transformed cell lines derived from type II cells. The most commonly used line, A459, is a neoplastic human cell derived from a nonsmall cell carcinoma expressing type II cell characteristics, such as surfactant apoprotein and lamellar bodies, and contains phospholipids for the production of surfactant (West et al., 1998). Investigations with A459 have focused on levels of proinflammatory transcription factors, AP-1 and NF-κB, and downstream proinflammatory cytokines and chemokines that orchestrate the inflammatory response. Treatment of A459 cells with collected PM_{10} activated NF-κB signaling and activated IL-8 transcription via histone acetylase activity directed at its promoter site (Jimenez et al., 2000; Gilmour et al., 2003). This inflammatory response was abrogated by antioxidant treatment.

AEC I also contributes to the generation of ROS. Techniques for the isolation of native type I cells from mice allow the dissection of PM-induced signaling pathways through comparisons with genetically modified strains. Such studies have demonstrated PM associated induction of AEC I apoptosis through a p53-mediated pathway (Urich et al., 2009). These findings have been confirmed *in vivo* following intratracheal instillation of $PM_{2.5}$ and shown to be dependent on mitochondrial electron transport-based ROS generation (Soberanes et al., 2009). The connection between ROS generation and induction of the apoptotic cascade appears to be through the redox-sensitive MAP3kinase effector apoptosis signal-regulating kinase 1 (ASK1) (Kyriakis and Avruch, 2001). ASK1 homeostasis is maintained through interaction with thioredoxin-1 with activation through ROS-mediated oxidation of cysteinyl sulfhydryl groups (Nadeau et al., 2009). PM-induced ROS oxidizes ASK1 and activates the stress activated protein kinase/c-Jun NH_2 terminal kinase (SAPK/JNK) pathway that leads to apoptosis *in vitro*. Interruption of this pathway prevents PM-induced apoptosis and alveolar leak *in vivo* (Soberanes et al., 2009). *In vitro* findings using an AEC line (A549) corresponded with similar *in vivo* results in a mouse and demonstrated a DEP-induced upregulation of matrix metalloprotease (MMP)-1, a collagenase involved in alveolar wall degradation via an oxidative pathway involving NAD(P)H oxidase and NOX4 (Amara et al., 2007).

Endothelial Cells

Recognition that NPs may be likely to enter the systemic circulation has stimulated research evaluating the direct effects of NPs on endothelium (Nemmar et al., 2002). The role that the endothelium plays in thrombus formation and atherogenesis has been focused on the context of health effects of PM. However, endothelial cells play a central role in the regulation of hemostasis through secretion of cytokines, chemokines, vasoactive mediators that balance constriction, and relaxation of smooth muscle cells that line the vasculature and coagulation signaling cascades.

A great deal of understanding of the pulmonary endothelial involvement in inflammation and coagulation has been derived from studies of acute lung injury associated with the ARDS. A somewhat simplified synopsis of these studies is that, under conditions of inflammatory stimulation, pulmonary microvascular endothelium switches from a predominantly anti-inflammatory phenotype that also inhibits coagulation to an activated state that promotes leukocyte adhesion and activation and initiates the coagulation cascade. A key component in this activation process appears to be the coincident activation of platelets. A central role for the LPS of gram-negative bacteria has been demonstrated for the induction of alveolar capillary inflammation in acute lung injury. LPS, in conjunction with circulating CD14, a receptor for LPS, interacts with endothelial cells through TLRs to upregulate leukocyte adhesion molecules including VCAM, ICAM, and E-selectin as well as stimulating the transport of P-selectin to the cell surface and the release of vWF from intracellular stores in Weibel–Palade bodies (Fig. 17.2). Local interactions with polymorphonuclear leukocytes (PMNs) and platelets can enhance this response by a variety of mechanisms including secretion of receptor cofactors and platelet–leukocyte–endothelial adhesion (for a detailed review, see Martinez and Zimmerman, 2007). Endothelial cells participate in the recruitment of leukocytes to injured tissue through surface expression of constitutive and cytokine inducible adhesion molecules (West et al., 1998). Expression of adhesion molecules, such as ICAM and VCAM, constitutively expressed on vascular endothelium and induced by LPS, supports the migration of leukocytes to injured tissues and are triggered by exposure to proinflammatory cytokines, such as IL-1β (Terry et al., 1993; Panes et al., 1995). Ultrafine particles were shown to induce microvascular endothelium production of ROS generation directly through NAD(P)H oxidase leading to activation of MAP kinase cascades and ultimately production of proinflammatory mediators such as IL-6 (Mo et al., 2009). Recent cellular studies have suggested a role for NPs in the induction of gene and protein levels of endothelial adhesion molecules ICAM, VCAM, and endothelial-leukocyte adhesion molecule (ELAM)-1 followed by

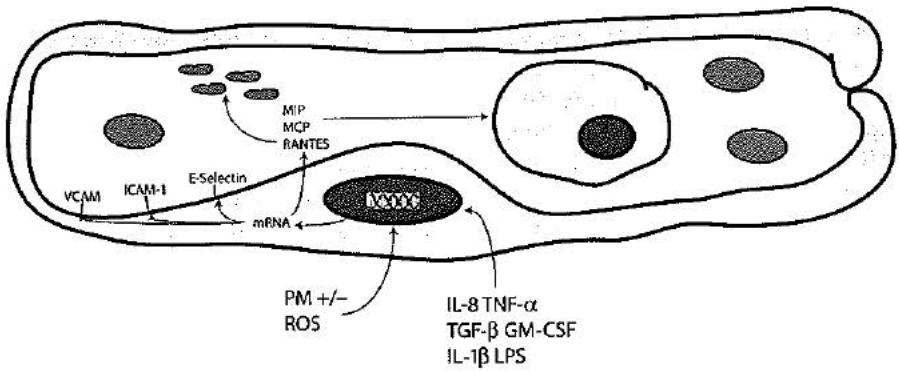

Figure 17.2 Diagram of the upregulation of endothelial adhesion molecules and proinflammatory activities that stimulate leukocytes and platelets in the pulmonary microvasculature that may be the key to both pulmonary and systemic inflammatory responses to inhaled particulate matter.

increased adhesion of activated monocytes (Oesterling et al., 2008). Exposure to DEPs has been shown to increase levels of IL-1β and ICAM-1 in mice (Takano et al., 2002). These measures are important because an increase in endothelial adhesiveness is involved in the development of vascular diseases such as atherosclerosis (Oesterling et al., 2008). Additionally, a variety of signaling molecules have been colocalized to endothelial caveolae, which are involved in the process of micropinocytosis. Signaling molecules involved in the regulation of vascular contractility and proliferation and interfacing with inflammatory stimuli include epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin receptors, protein kinase C (PKC), protein kinase A (PKA), adenyl cyclase, TGF-β family receptors, intermediates of MAP kinase signal transduction, and eNOS (Ramos et al., 2007).

The function of the endothelium as a barrier has been investigated in the umbilical vein endothelium (Nadadur et al., 2009). In endothelium treated with residual oil fly ash, multiple genes associated with ion transport as well as intracellular signal regulation and extracellular matrix were identified that are likely to influence endothelial barrier function (Nadadur et al., 2009). While relatively little information on the potential direct effects of particulate exposure for endothelial barrier function has been published, several ROS-mediated signaling pathways regulate endothelial cytoskeleton and/or junctional protein assembly. ROS effects on cytoskeletal contractility include inhibition of myosin light-chain kinase, p38 MAP kinase-induced phosphorylation of the actin binding heat shock protein HSP27, and activation of RhoGTPases (Boueiz and Hassoun, 2009). ROS also influences the integrity of endothelial cell–cell junctions, specifically by activating tyrosine kinases that modify components of the adherens junctions (Boueiz and Hassoun, 2009) (Fig. 17.3).

Endothelium of the pulmonary vasculature is a major target of injury by oxidants but can also contribute to reactive oxygen species (ROS) levels (West et al., 1998). Vascular endothelium is a known source of ROS production via mitochondria; xanthine oxidase; arachidonic metabolizing enzymes; heme-containing peroxidases; NAD(P)H oxidases, including NOX2 and NOX4 isoforms; and nitric oxide synthases (NOS) (West et al., 1998). In the case of NOX2, the phosphorylation of $p47^{phox}$ leads to membrane transport and activation of the complex. Whether this serves an antimicrobial action or as a signaling mechanism remains uncertain. It is now increasingly accepted that NOX-derived ROS provides a spatially and temporally limited mechanism for compartmentally restricted signaling (Terada, 2006). *In vitro* experiments with mouse pulmonary microvascular cells demonstrate activation of the NOX2 complex and ROS generation that is associated with MAP kinase pathways (Mo et al., 2009). Vascular endothelial growth factor (VEGF)-mediated activation of NOX2-associated complexes is involved in regulation of focal adhesion contacts, formation of lamellipodae, and regulation of intercellular junction integrity (Ushio-Fukai, 2007). This is largely mediated through NOX2-derived ROS-inhibiting tyrosine phosphatases (Ushio-Fukai, 2009). Endothelial cells express NOX4 in the endoplasmic reticulum (ER) and NOX4-mediated ROS generation drives V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (K-ras) activation leading to endothelial protective responses to chemical stressors (Wu et al., 2010). NOX4 also plays an important role in angiogenesis, again through VEGF signaling pathways. ROS produced by activation of NOX4 subsequent to VEGF signaling

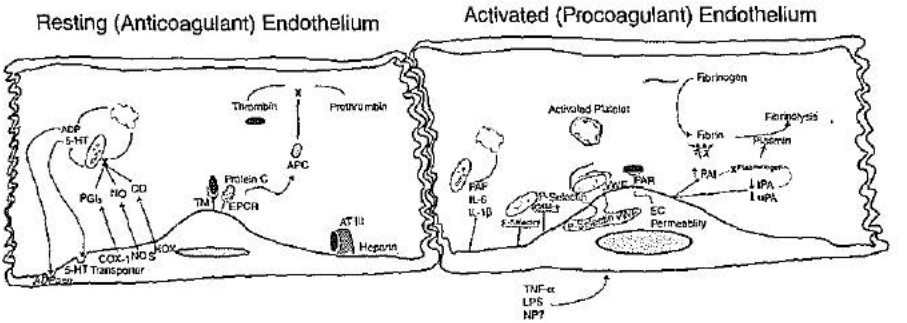

Figure 17.3 Regulation of the coagulant system by resting or activated endothelium. Resting endothelium inhibits platelet activation through synthesis of NO, CO, and PGI_2 as well as uptake or metabolism of platelet-derived 5-HT and ADP. Normal endothelium also inhibits thrombin activity through binding to thrombomodulin (TM), activation of protein C (APC) through collaborative interactions of TM with endothelial cell protein C receptor (EPCR), and heparin-enhanced binding of thrombin by antithrombin III (AT III). Endothelium activated by endotoxin (LPS) or TNF-α and possibly nanoparticles switches to a procoagulant state with upregulation of E-selectin, ICAM-1, and expression of platelet endothelial cell adhesion molecule (PECAM) and vWF from intracellular stores in Weibel–Palade bodies. Endothelial cell (EC) surface receptors bind platelets that are further activated by EC-derived cytokines, such as IL-6, platelet activating factor (PAF), and IL-1α. Activated and bound platelets externalize platelet granules and bind fibrinogen. These primed platelets can be released to the circulation where they may enhance systemic coagulant responses. Activated EC also modulates regulation of fibrin degradation by inhibiting plasminogen activator through decreased production of tissue plasminogen activator (tPA) and urokinase (uPA) as well as secretion of plasminogen activator inhibitor (PAI).

inhibits protein tyrosine phosphatases thus enhancing phosphorylation of PDGF and extracellular signal-regulated kinase (ERK) 1 and 2 (Datla et al., 2007). Whether overactivation of these endogenous signaling systems leads to oxidative stress and whether PM exposure in the pulmonary microvasculature activates these systems remains undetermined.

Endothelial cells participate in the regulation of coagulation cascades. Coagulant cascades are facilitated by the initial activation of platelets triggered by damaged vascular endothelium followed by activation of enzymatic cascades to produce fibrin to strengthen the clot and facilitate cellular repair. If these cascades are disrupted, an increased risk for clotting, or thrombosis, exists. Mechanistically, this disruption may be facilitated by exposure to ambient particles as physiological studies have revealed changes in several key coagulant mediators (Rueckerl et al., 2006; Bigert et al., 2008). Upregulation of the procoagulant activity of tissue factor (TF) and correlative downregulation of the thrombolytic activity tPA demonstrated procoagulant responses in PM-treated umbilical vein endothelium (Gilmour et al., 2005). TF is also elicited from cultured bronchial epithelium and monocytes and vascular smooth muscle cells treated with collected ambient PM (Sun et al., 2008). A principal shift in endothelial regulation of the coagulation cascade is a shift from synthesis of the anticoagulant prostacylin I_2 (PGI_2) to procoagulant thromboxane A2 (TBX2). These mediators are synthesized from arachidonic acid precursors by the

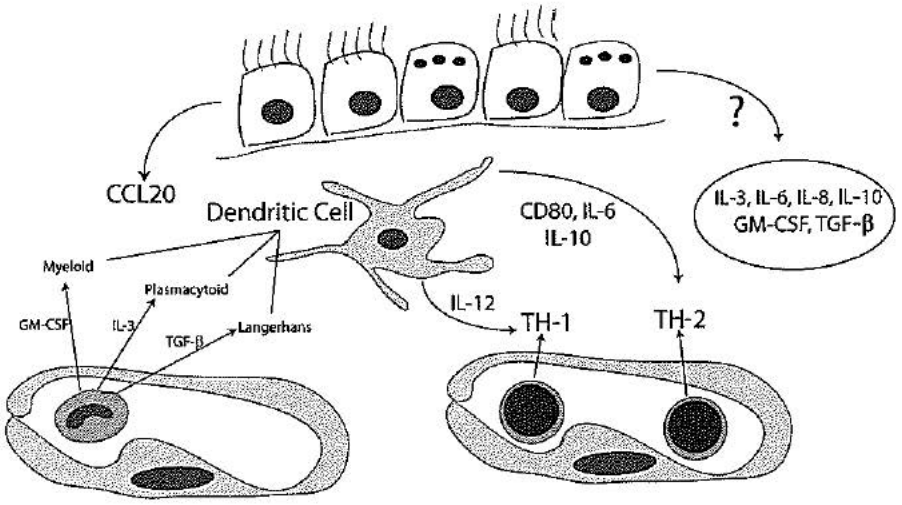

Figure 17.4 Diagram of interactions between epithelial cell-derived signals and dendritic cell responses attracting and inducing TH-1 lymphocytes and their associated immunomodulatory IFN-γ secretion or proallergenic TH-2 responses.

COX enzymes COX-1 and COX-2. Several additional factors interface in the regulation of coagulation including vWF expression and secretion, thrombomodulin, TF pathway inhibitor, antithrombin III, and proteins C and S. These proteins regulate the activation of the coagulation cascade as well as endothelial signaling through the thrombin-activated, protease-activated receptor system (Fig. 17.4).

Research has demonstrated a role for particles in the alteration of the activity of vasoregulatory substances such as NO, endothelial-derived relaxing factor (EDRF), and endothelin-1 (ET-1). Principal among these are alterations in NO homeostasis and synthesis of ET-1 (Brook et al., 2002; Urch et al., 2005; Brook, 2008). Children exposed to ambient PM demonstrate elevated plasma ET-1 and mean pulmonary arterial pressure (Calderon-Garciduenas et al., 2007). Inhalation of urban PM has been demonstrated to increase vascular tone in small pulmonary arterioles (Batalha et al., 2002). This process appears, in part, to be a consequence of altered NO-mediated vasorelaxation. Direct PM treatment of isolated pulmonary artery segments and segments isolated from PM-instilled rats results in decreased NO-dependent relaxation in response to acetylcholine (Courtois et al., 2008).

Vasoregulatory mediators are released by endothelial cells. NO is an important endogenous regulator of pulmonary vascular tone. NO is synthesized and released by endothelial cells, causing relaxation of vascular smooth muscle cells and inhibiting platelet activation. NO sets basal vascular tone and contributes to hypertension when inhibited. Biosynthesis and release of NO is known to be impaired at an early stage in a variety of vascular disorders (West et al., 1998). NO plays a role in the accumulation of cyclic guanosine monophosphate (cGMP) leading to smooth muscle relaxation, but it is uncertain how indicative measurement of cGMP levels are for NO production by endothelial cells (West et al., 1998). Vasodilator drugs act on membrane-bound G-protein-coupled receptors on the vascular endothelium and

stimulate calcium influx and NOS (eNOS) activation. This increases local NO levels to act on adjacent smooth muscle cells causing vasodilation and increased blood flow.

In addition to NO, endothelins are proteins involved in vasoconstriction through constriction of blood vessels and elevations in blood pressure. Overproduction of endothelin (ET-1) in the lungs may cause pulmonary hypertension when the balance between constrictors, such as endothelin, and dilators, such as NO and prostacyclins, is disrupted. The endothelium regulates local vascular tone and integrity through the coordinated release of vasoactive molecules. Secretion of ET-1 from the endothelium signals vasoconstriction and influences local cellular growth and survival. ET-1 has been implicated in the development and progression of vascular disorders, such as atherosclerosis and hypertension. Endothelial cells upregulate ET-1 in response to hypoxia, oxidized LDL, proinflammatory cytokines, and bacterial toxins. Initial studies on the ET-1 promoter provided some of the earliest mechanistic insight into endothelial-specific gene regulation. Numerous studies have since provided valuable understanding of ET-1 promoter regulation under basal and activated cellular states.

Lung capillaries express a high level of angiotensin-converting enzyme—sufficient to clear all circulating bradykinin and to activate all angiotensin I to angiotensin II. They also actively synthesize serotonin and transport it to vascular smooth muscle cells. Similarly, endothelial cells are prominent sources for endothelin synthesis and its metabolism to its active ET-1 form. Since ET-1 is primarily synthesized and partially cleared in the lungs, alterations in its secretion provide a clear link between pulmonary changes induced by PM within the pulmonary vasculature that may contribute to systemic responses. Lung tissue damaged due to acute and chronic inflammation and cellular damage reduces ET-1 production to provide adequate balance to vasoconstrictors. Rats exposed to inhaled urban PM had increased gene expression of lung endothelin system genes, which are the primary source of circulating ET-1, and detectably increased circulating ET-1 and blood pressure (Thomson et al., 2004, 2005, 2006). This increase was attributed to the soluble fraction of urban particulates and was not induced by DEPs. Thomson and colleagues provided extensive research supporting a role for endothelins in the mechanism for induction of cardiovascular effects by inhaled particles.

UNIFYING HYPOTHESES

Pulmonary inflammation induced by particulates has important consequences for airway-associated diseases, such as asthma and chronic bronchitis, and as a stimulus for systemic proinflammatory and procoagulant effects that provide a mechanistic explanation for PM-associated CVD, such as myocardial infarction and atherogenesis. The responses of individual cells, as reviewed above, leads to interactive signaling of diverse and often divergent inflammatory pathways. These include both innate and immune defense systems with the interplay between them potentially directing alternative disease outcomes (Fig. 17.1).

In the case of airway diseases, the interface between airway epithelial cell responses and the immune system is predicated on selective induction of TH-1- or TH-2-like responses and the recruitment of either neutrophils that will drive bron-

chitis or dendritic cells that influence airway immune responses and asthma. Both pathways can stimulate mucus hypersecretion through diverse signals. The interaction between PM-generated ROS, receptor activation, differential TLR signaling, and resultant patterns of bronchial epithelial cytokine secretion is important in characterizing differential inflammatory and immune responses for particulates from differing sources. Future studies should contrast characteristic TH-1 patterns of secretion of IL-12, IL-18, TNF-α, and IFN-γ with TH-2 responses, such as IL-4, IL-5, and IL-13. These effector cell signals should be interpreted in context of inflammatory cell recruitment patterns and activation of receptor (epithelial) cell signaling through TLR and growth factor activation and secretion of IL-6, IL-8, TNF-α, and CCL20.

Differential dendritic cell activation and consequences on TH-1 or TH-2 immunomodulatory responses may be the key in the relationship between inhaled PM and asthma or bronchitis. Dendritic cells (DC) recruited from monocytes (myeloid DC), the plasmacytoid series, or induced to become Langerhans-type cells drive differing responses relative to interferon secretion and TH induction (Upham and Stumbles, 2003). Recruitment and differentiation of differing dendritic cell populations may depend on the epithelial cell-derived cytokine milieu. Similarly, epithelial cell-derived signals may direct dendritic cell responses attracting and inducing TH-1 lymphocytes and their associated immunomodulatory IFN-γ secretion or proallergenic TH-2 responses (Fig. 17.4).

PM influences on the alveolar wall have the potential to influence alveolar integrity, edema formation, and perhaps interstitial matrix synthesis associated with fibrosis. The alveolar capillary network also seems a likely region for the secretion of cytokines and activation of circulating inflammatory cells including platelets. An understanding of predicted patterns of deposition and clearance for particles with a variety of physicochemical characteristics leads to the development of hypotheses regarding signaling and response patterns. Soluble cytokines secreted by AMs and epithelial cells may interact directly through endothelial cell receptors or even be modulated by extracellular matrix. Transport of particles through the alveolar wall to directly interact with microvascular endothelium is highly plausible, especially for those in the ultrafine size range. This seems to be the most logical site for the activation of the circulating elements of blood, especially given the inherent metabolic capacity of alveolar endothelium relative to inflammatory mediators. Upregulation of endothelial adhesion molecules and secretion of proinflammatory activities that stimulate leukocytes and platelets (Fig. 17.2) in the pulmonary microvasculature may be the key to both pulmonary and systemic inflammatory responses to inhaled PM. Specific activities of importance for priming of platelets and activation of monocytes include the expression of endothelial cell adhesion molecules, such as ICAM-1, E-selectin, P-selectin, and VCAM-1, and the secretion of proinflammatory cytokines activating circulating leukocytes and platelets, such as MIP-1, monocyte chemotactic protein (MCP)-1, IL-1α, RANTES, ET-1, and TF. Additionally, alterations in endothelial-derived anti-inflammatory activities of PGI_2, inducible nitric oxide synthase (iNOS), tPA, and other thromboregulatory proteins may be important in the complex response to particulate-induced cardiovascular effects.

SUMMARY AND ONGOING RESEARCH QUESTIONS

The cytokine milieu of pulmonary airways and alveolar spaces is a highly interconnected network of epithelial-, endothelial-, and leukocyte-derived activities with potential to influence both pulmonary and systemic responses to inhaled particles. These responses involve much of particle toxicology, including deposition patterns, size, composition, copollutant contamination and genetic variation in the responses of individuals. Individual cells use many of the mechanisms evolved in response to infectious agents and thus include mediators affecting both nonspecific and immune defense systems. Recent evidence provides new clues connecting the chemical–biological interactions between diverse source particles and innate stress response signaling pathways, particularly in relation to oxidant response elements. The interface between pulmonary inflammation, pulmonary microvascular responses, and systemic inflammation is an area for continuing investigation. The unique chemical and physical characteristics of ambient and engineered NPs present a challenge toward understanding the toxicity and downstream health effects.

ACKNOWLEDGEMENTS

The background literature research for this chapter has contributed to conceptual development of original research funded by grants from the National Institute of Occupational Safety and Health (U07/CCU906162), the U.S. Environmental Protection Agency (RD-832414 and R826246), and the National Institute of Environmental Health Sciences (RC1 ES 018232). The authors thank Suzette Smiley-Jewell for editorial support. The authors have sole responsibility for the writing and content of the chapter. The contents of this review may not necessarily reflect the views of the funding organizations.

REFERENCES

Albertine, K.H., Wiener Kronish, J.P., Roos, P.J., Staub, N.C. 1982. Structure blood supply and lymphatic vessels of the sheeps visceral pleura. *Am. J. Anat.* 165:277 294.

Alexis, N.E., Lay, J.C., Zeman, K., Bennett, W.E., Peden, D.B., Soukup, J.M., Devlin, R.B., Becker, S. 2006. Biological material on inhaled coarse fraction particulate matter activates airway phagocytes in vivo in healthy volunteers. *J. Allergy Clin. Immunol.* 117:1396 1403.

Amara, N., Bachoual, R., Desmard, M., Golda, S., Guichard, C., Lanone, S., Aubier, M., Ogier Denis, E., Boczkowski, J. 2007. Diesel exhaust particles induce matrix metalloprotease 1 in human lung epithelial cells via a NADP(H) oxidase/NOX4 redox dependent mechanism. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 293:L170 L181.

Araujo, J.A., Barajas, B., Kleinman, M., Wang, X.P., Bennett, B.J., Gong, K.W., Navab, M., Harkema, J., Sioutas, C., Lusis, A.J., Nel, A.E. 2008. Ambient particulate pollutants in the ultrafine range promote early atherosclerosis and systemic oxidative stress. *Circ. Res.* 102:589 596.

Bailey, K.L., Poole, J.A., Mathisen, T.L., Wyatt, T.A., Von Essen, S.G., Romberger, D.J. 2008. Toll-like receptor 2 is upregulated by hog confinement dust in an IL 6-dependent manner in the airway epithelium. *Am. J. Physiol. Lung Cell Mol. Physiol.* 294:L1049 L1054.

Barnes, P.J., Liu, S.F. 1995. Regulation of pulmonary vascular tone. *Pharmacol. Rev.* 47:87–131.

Bartoli, C.R., Wellenius, G.A., Diaz, E.A., Lawrence, J., Coull, B.A., Akiyama, I., Lee, L.M., Okabe, K., Verrier, R.L., Godleski, J.J. 2009. Mechanisms of inhaled fine particulate air pollution-induced arterial blood pressure changes. *Environ. Health Perspect.* 117:361–366.

Batalha, J.R.F., Saldiva, P.H.N., Clarke, R.W., Coull, B.A., Stearns, R.C., Lawrence, J., Murthy, G.G.K., Koutrakis, P., Godleski, J.J. 2002. Concentrated ambient air particles induce vasoconstriction of small pulmonary arteries in rats. *Environ. Health Perspect.* 110:1191–1197.

Becker, S., Soukup, J.M., Gilmour, M.I., Devlin, R.B. 1996. Stimulation of human and rat alveolar macrophages by urban air particulates: Effects on oxidant radical generation and cytokine production. *Toxicol. Appl. Pharmacol.* 141:637–648.

Becker, S., Clapp, W.A., Quay, J., Frees, K.L., Koren, H.S., Schwartz, D.A. 1999. Compartmentalization of the inflammatory response to inhaled grain dust. *Am. J. Respir. Crit. Care Med.* 160:1309–1318.

Becker, S., Soukup, J.M., Sioutas, C., Cassee, F.R. 2003. Response of human alveolar macrophages to ultrafine, fine, and coarse urban air pollution particles. *Exp. Lung Res.* 29:29–44.

Becker, S., Dailey, L.A., Soukup, J.M., Grambow, S.C., Devlin, R.B., Huang, Y.C. 2005. Seasonal variations in air pollution particle-induced inflammatory mediator release and oxidative stress. *Environ. Health Perspect.* 113:1032–1038.

Bigert, C., Alderling, M., Svartengren, M., Plato, N., de Faire, U., Gustavsson, P. 2008. Blood markers of inflammation and coagulation and exposure to airborne particles in employees in the Stockholm underground. *Occup. Environ. Med.* 65:655–658.

van den Bogaard, E.H.J., Dailey, L.A., Thorley, A.J., Tetley, T.D., Forbes, B. 2009. Inflammatory response and barrier properties of a new alveolar type 1-like cell line (TT1). *Pharm. Res.* 26:1172–1180.

Bonvallot, V., Baeza-Squiban, A., Baulig, A., Brulant, S., Boland, S., Muzeau, F., Barouki, R., Marano, F. 2001. Organic compounds from diesel exhaust particles elicit a proinflammatory response in human airway epithelial cells and induce cytochrome p450 1A1 expression. *Am. J. Respir. Cell Mol. Biol.* 25:515–521.

Borok, Z., Liebler, J.M., Li, X., Zhou, B., Foster, M.J., Kim, K.-J., Crandall, E.D. 2002a. Evidence for participation of type I cells in alveolar sodium homeostasis. *FASEB J.* 16:A402.

Borok, Z., Liebler, J.M., Lubman, R.L., Foster, M.J., Zhou, B., Li, X., Zabski, S.M., Kim, K.-J., Crandall, E.D. 2002b. Na transport proteins are expressed by rat alveolar epithelial type I cells. *Am. J. Physiol.* 282:L599–L608.

Borok, Z., Sandoval, A.J., Zhou, B., Ma, D., Liebler, J.M., Willis, B.C., Kim, K.J., Crandall, E.D. 2002c. Differential expression of claudins in alveolar epithelial cells. *Mol. Biol. Cell.* 13:498a.

Boueiz, A., Hassoun, P.M. 2009. Regulation of endothelial barrier function by reactive oxygen and nitrogen species. *Microvasc. Res.* 77:26–34.

Brandenberger, C., Rothen-Rutishauser, B., Blank, F., Gehr, P., Muhlfeld, C. 2009. Particles induce apical plasma membrane enlargement in epithelial lung cell line depending on particle surface area dose. *Respir. Res.* 10:15.

Brauner, E.V., Moller, P., Barregard, L., Dragsted, L.O., Glasius, M., Wahlin, P., Vinzents, P., Raaschou-Nielsen, O., Loft, S. 2008. Exposure to ambient concentrations of particulate air pollution does not influence vascular function or inflammatory pathways in young healthy individuals. *Part. Fibre Toxicol.* 5:9.

Brook, R.D. 2008. Cardiovascular effects of air pollution. *Clin. Sci. (Lond.)* 115:175–187.

Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S., Silverman, F. 2002. Fine particulate air pollution and ozone inhalation causes acute arterial vasoconstriction in healthy adults. *J. Am. Coll. Cardiol.* 39:219A.

Brown, D.M., Donaldson, K., Borm, P.J., Schins, R.P., Dehnhardt, M., Gilmour, P., Jimenez, L.A., Stone, V. 2004. Calcium and ROS-mediated activation of transcription factors and TNF-alpha cytokine gene expression in macrophages exposed to ultrafine particles. *Am. J. Physiol.* 286:L344–L353.

Buzea, C., Pacheco, I.I., Robbie, K. 2007. Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases* 2:MR17–MR71.

Calderon-Garciduenas, L., Vincent, R., Mora-Tiscareno, A., Franco-Lira, M., Henriquez-Roldan, C., Barragan-Mejia, G., Garrido-Garcia, L., Camacho-Reyes, L., Valencia-Salazar, G., Paredes, R., Romero, L., Osnaya, H., Villarreal-Calderon, R., Torres-Jardon, R., Hazucha, M.J., Reed, W. 2007.

Elevated plasma endothelin-1 and pulmonary arterial pressure in children exposed to air pollution. *Environ. Health Perspect.* 115:1248–1253.

Chatterjee, S., Al-Mehdi, A.-B., Levitan, I., Stevens, T., Fisher, A.B. 2003. Shear stress increases expression of a KATP channel in rat and bovine pulmonary vascular endothelial cells. *Am. J. Physiol.* 285: C959–C967.

Chen, J., Chen, Z., Chintagari, N.R., Bhaskaran, M., Jin, N., Narasaraju, T., Liu, L. 2006. Alveolar type I cells protect rat lung epithelium from oxidative injury. *J. Physiol. (Oxford)* 572:625–638.

Cho, A.K., Sioutas, C., Miguel, A.H., Kumagai, Y., Schmitz, D.A., Singh, M., Eiguren-Fernandez, A., Froines, J.R. 2005. Redox activity of airborne particulate matter at different sites in the Los Angeles Basin. *Environ. Res.* 99:40–47.

Churg, A., Xie, C.S., Wang, X.S., Vincent, R., Wang, R.D. 2005. Air pollution particles activate NF-kappa B on contact with airway epithelial cell surfaces. *Toxicol. Appl. Pharmacol.* 208:37–45.

Clift, M.J.D., Rothen-Rutishauser, B., Brown, D.M., Duffin, R., Donaldson, K., Proudfoot, L., Guy, K., Stone, V. 2008. The impact of different nanoparticle surface chemistry and size on uptake and toxicity in a murine macrophage cell line. *Toxicol. Appl. Pharmacol.* 232:418–427.

Courtois, A., Andujar, P., Ladeiro, Y., Baudrimont, I., Delannoy, E., Leblais, V., Begueret, H., Galland, M.A.B., Brochard, P., Marano, F., Marthan, R., Muller, B. 2008. Impairment of NO-dependent relaxation in intralobar pulmonary arteries: Comparison of urban particulate matter and manufactured nanoparticles. *Environ. Health Perspect.* 116:1294–1299.

Cozzi, E., Wingard, C.J., Cascio, W.E., Devlin, R.B., Miles, J.J., Bofferding, A.R., Lust, R.M., Van Scott, M.R., Henriksen, R.A. 2007. Effect of ambient particulate matter exposure on hemostasis. *Transl. Res.* 149:324–332.

Datla, S.R., Peshavariya, H., Dusting, G.J., Mahadev, K., Goldstein, B.J., Jiang, F. 2007. Important role of Nox4 type NADPH oxidase in angiogenic responses in human microvascular endothelial cells in vitro. *Arteriosclerosis Thrombosis and Vascular Biology* 27:2319–2324.

David, J., Wilkinson, L.J., Griffiths, G.D. 2009. Inflammatory gene expression in response to sub-lethal ricin exposure in Balb/c mice. *Toxicology* 264:119–130.

Deguillaume, L., Leriche, M., Desboeufs, K., Mailhot, G., George, C., Chaumerliac, N. 2005. Transition metals in atmospheric liquid phases: Sources, reactivity, and sensitive parameters. *Chem. Rev.* 105:3388–3431.

Delfino, R.J., Staimer, N., Tjoa, T., Polidori, A., Arhami, M., Gillen, D.L., Kleinman, M.T., Vaziri, N.D., Longhurst, J., Zaldivar, F., Sioutas, C. 2008. Circulating biomarkers of inflammation, antioxidant activity, and platelet activation are associated with primary combustion aerosols in subjects with coronary artery disease. *Environ. Health Perspect.* 116:898–906.

Delfino, R.J., Staimer, N., Tjoa, T., Gillen, D.L., Polidori, A., Arhami, M., Kleinman, M.T., Vaziri, N.D., Longhurst, J., Sioutas, C. 2009. Air pollution exposures and circulating biomarkers of effect in a susceptible population: Clues to potential causal component mixtures and mechanisms. *Environ. Health Perspect.* 117:1232–1238.

Dockery, D.W. 2001. Epidemiologic evidence of cardiovascular effects of particulate air pollution. *Environ. Health Perspect.* 109:483–486.

Eaton, D.C., Helms, M.N., Koval, M., Bao, H.F., Jain, L. 2009. The contribution of epithelial sodium channels to alveolar function in health and disease. *Annu. Rev. Physiol.* 71:403–423.

Effros, R.M., Parker, J.C. 2009. Pulmonary vascular heterogeneity and the Starling hypothesis. *Microvasc. Res.* 78:71–77.

Elder, A.C., Gelein, R., Azadniv, M., Frampton, M., Finkelstein, J., Oberdorster, G. 2004. Systemic effects of inhaled ultrafine particles in two compromised, aged rat strains. *Inhal. Toxicol.* 16:461–471.

Ferin, J. 1994. Pulmonary retention and clearance of particles. *Zentralbl. Hyg. Umweltmed.* 195:148.

Ferin, J., Oberdorster, G., Penney, D.P. 1992. Pulmonary retention of ultrafine and fine particles in rats. *Am. J. Respir. Cell Mol. Biol.* 6:535–542.

Forman, H.J., Torres, M. 2002. Reactive oxygen species and cell signaling: Respiratory burst in macrophage signaling. *Am. J. Respir. Crit. Care Med.* 166:S4–S8.

Frampton, M.W., Stewart, J.C., Oberdoerster, G., Morrow, P.E., Chalupa, D., Pietropaoli, A.P., Frasier, L.M., Speers, D.M., Cox, C., Huang, L.-S., Utell, M.J. 2006. Inhalation of ultrafine particles alters blood leukocyte expression of adhesion molecules in humans. *Environ. Health Perspect.* 114:51–58.

Gardner, S.Y., McGee, J.K., Kodavanti, U.P., Ledbetter, A., Everitt, J.I., Winsett, D.W., Doerfler, D.L., Costa, D.L. 2004. Emission-particle-induced ventilatory abnormalities in a rat model of pulmonary hypertension. *Environ. Health Perspect.* 112:872–878.

Gilmour, P.S., Rahman, I., Donaldson, K., MacNee, W. 2003. Histone acetylation regulates epithelial IL-8 release mediated by oxidative stress from environmental particles. *Am. J. Physiol. Lung Cell Mol. Physiol.* 284:L533–L540.

Gilmour, P.S., Morrison, E.R., Vickers, M.A., Ford, I., Ludlam, C.A., Greaves, M., Donaldson, K., MacNee, W. 2005. The procoagulant potential of environmental particles (PM10). *Occup. Environ. Med.* 62:164–171.

Gojova, A., Guo, B., Kota, R.S., Rutledge, J.C., Kennedy, I.M., Barakat, A.I. 2007. Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: Effect of particle composition. *Environ. Health Perspect.* 115:403–409.

Green, R.M., Hodges, N.J., Chipman, J.K., O'Donovan, M.R., Graham, M. 2008. Reactive oxygen species from the uncoupling of human cytochrome P450 1B1 may contribute to the carcinogenicity of dioxin-like polychlorinated biphenyls. *Mutagenesis* 23:457–463.

Grubor, B., Meyerholz, D.K., Ackermann, M.R. 2006a. Collectins and cationic antimicrobial peptides of the respiratory epithelia. *Vet. Pathol.* 43:595–612.

Grubor, B., Meyerholz, D.K., Lazic, T., DeMacedo, M.M., Derscheid, R.J., Hostetter, J.M., Gallup, J.M., DeMartini, J.C., Ackermann, M.R. 2006b. Regulation of surfactant protein and defensin mRNA expression in cultured ovine type II pneumocytes by all-trans retinoic acid and VEGF. *Int. J. Exp. Pathol.* 87:393–403.

Huang, Y.C.T., Li, Z.W., Harder, S.D., Soukup, J.M. 2004. Apoptotic and inflammatory effects induced by different particles in human alveolar macrophages. *Inhal. Toxicol.* 16:863–878.

Hussain, A.A. 1989. Mechanism of nasal absorption of drugs. *Prog. Lin. Biol. Res.* 292:261–272.

Imrich, A., Ning, Y.Y., Koziel, H., Coull, B., Kobzik, L. 1999. Lipopolysaccharide priming amplifies lung macrophage tumor necrosis factor production in response to air particles. *Toxicol. Appl. Pharmacol.* 159:117–124.

Imrich, A., Ning, Y.Y., Lawrence, J., Coull, B., Gitin, E., Knutson, M., Kobzik, L. 2007. Alveolar macrophage cytokine response to air pollution particles: Oxidant mechanisms. *Toxicol. Appl. Pharmacol.* 218:256–264.

Inoue, K.-I., Takano, H., Yanagisawa, R., Hirano, S., Sakurai, M., Shimada, A., Yoshikawa, T. 2006. Effects of airway exposure to nanoparticles on lung inflammation induced by bacterial endotoxin in mice. *Environ. Health Perspect.* 114:1325–1330.

Ishii, H., Hayashi, S., Hogg, J.C., Fujii, T., Goto, Y., Sakamoto, N., Mukae, H., Vincent, R., van Eeden, S.F. 2005. Alveolar macrophage-epithelial cell interaction following exposure to atmospheric particles induces the release of mediators involved in monocyte mobilization and recruitment. *Respir. Res.* 6:12.

Jimenez, L.A., Thompson, J., Brown, D.A., Rahman, I., Antonicelli, F., Duffin, R., Drost, E.M., Hay, R.T., Donaldson, K., MacNee, W. 2000. Activation of NF-kappa B by PM10 occurs via an iron-mediated mechanism in the absence of I kappa B degradation. *Toxicol. Appl. Pharmacol.* 166:101–110.

Kobzik, L., Frevert, C., Imrich, A., Sioutas, C. 1995. Role of scavenger receptors in lung macrophage binding of environmental particles. *FASEB J.* 9:A959.

Kodavanti, U.P., Jackson, M.C., Ledbetter, A.D., Richards, J.R., Gardner, S.Y., Watkinson, W.P., Campen, M.J., Costa, D.L. 1999. Lung injury from intratracheal and inhalation exposures to residual oil fly ash in a rat model of monocrotaline-induced pulmonary hypertension. *J. Toxicol. Environ. Health A* 57:543–563.

Koyama, S., Sato, E., Masubuchi, T., Takamizawa, A., Kubo, K., Nagai, S., Izumi, T. 1998a. Alveolar type II-like cells release G-CSF as neutrophil chemotactic activity. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 275:L687–L693.

Koyama, S., Sato, E., Nomura, H., Kubo, K., Miura, M., Yamashita, T., Nagai, S., Izumi, T. 1998b. Bradykinin stimulates type II alveolar cells to release neutrophil and monocyte chemotactic activity and inflammatory cytokines. *Am. J. Pathol.* 153:1885–1893.

Kreyling, W.G., Semmler, M., Erbe, F., Mayer, P., Takenaka, S., Schulz, H., Oberdorster, G., Ziesenis, A. 2002. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J. Toxicol. Environ. Health A* 65:1513–1530.

Kreyling, W.G., Semmler-Behnke, M., Seitz, J., Scymczak, W., Wenk, A., Mayer, P., Takenaka, S., Oberdörster, G. 2009. Size dependence of the translocation of inhaled iridium and carbon nanoparticle aggregates from the lung of rats to the blood and secondary target organs. *Inhal. Toxicol.* 21: 55–60.

Krieglstein, C.F., Granger, D.N. 2001. Adhesion molecules and their role in vascular disease. *Am. J. Hypertens.* 14:44S–54S.

Kyriakis, J.M., Avruch, J. 2001. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol. Rev.* 81:807–869.

Lambeth, J.D., Kawahara, T., Diebold, B. 2007. Regulation of Nox and Duox enzymatic activity and expression. *Free Radic. Biol. Med.* 43:319–331.

Lauer, F.T., Mitchell, L.A., Bedrick, E., McDonald, J.D., Lee, W.Y., Li, W.W., Olvera, H., Amaya, M.A., Berwick, M., Gonzales, M., Currey, R., Pingitore, N.E., Burchiel, S.W. 2009. Temporal-spatial analysis of US-Mexico border environmental fine and coarse PM air sample extract activity in human bronchial epithelial cells. *Toxicol. Appl. Pharmacol.* 238:1–10.

Lucking, A.J., Lundback, M., Mills, N.L., Faratian, D., Barath, S.L., Pourazar, J., Cassee, F.R., Donaldson, K., Boon, N.A., Badimon, J.J., Sandstrom, T., Blomberg, A., Newby, D.E. 2008. Diesel exhaust inhalation increases thrombus formation in man. *Eur. Heart J.* 29:3043–3051.

Lundborg, M., Bouhafs, R., Gerde, P., Ewing, P., Camner, P., Dahlen, S.E., Jarstrand, C. 2007. Aggregates of ultrafine particles modulate lipid peroxidation and bacterial killing by alveolar macrophages. *Environ. Res.* 104:250–257.

Madl, A.K., Wilson, D.W., Segall, H.J., Pinkerton, K.E. 1998. Alteration in lung particle translocation, macrophage function, and microfilament arrangement in monocrotaline-treated rats. *Toxicol. Appl. Pharmacol.* 153:28–38.

Mark Evans, A., Ward, J.P. 2009. Hypoxic pulmonary vasoconstriction—Invited article. *Adv. Exp. Med. Biol.* 648:351–360.

Martinez, M.L., Zimmerman, G.A. 2007. The endothelium in acute respiratory distress syndrome. In: Aird, W., ed. *Endothelial Biomedicine.* New York: Cambridge University Press, pp. 1178–1192.

McLaughlin, R.F.J. 1983. Bronchial artery distribution in various mammals and in humans. *Am. Rev. Respir. Dis.* 128:S57–S58.

Message, S.D., Johnston, S.L. 2004. Host defense function of the airway epithelium in health and disease: Clinical background. *J. Leukoc. Biol.* 75:5–17.

Mills, N.L., Robinson, S.D., Gonzalez, M., MacNee, W., Cassee, F.R., Blomberg, A., Sandstrom, T., Donaldson, K., Boon, N.A., Tornqvist, H. 2005a. Inhalation of particulate air pollution impairs vascular and fibrinolytic function in man. *Circulation* 112:U348.

Mills, N.L., Tornqvist, H., Robinson, S.D., Gonzalez, M., Darnley, K., MacNee, W., Boon, N.A., Donaldson, K., Blomberg, A., Sandstrom, T., Newby, D.E. 2005b. Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. *Circulation* 112:3930–3936.

Mills, N.L., Tornqvist, H., Robinson, S.D., Gonzalez, M.C., Soderberg, S., Sandstrom, T., Blomberg, A., Newby, D.E., Donaldson, K. 2007. Air pollution and atherothrombosis. *Inhal. Toxicol.* 19: 81–89.

Miserocchi, G. 2007. Lung interstitial pressure and structure in acute hypoxia. In: Roach, R.C., Wagner, P.D., Hackett, P.H., eds. *Advances in Experimental Medicine and Biology.* New York: Springer, pp. 141–157.

Mo, Y., Wan, R., Chien, S., Tollerud, D.J., Zhang, Q. 2009. Activation of endothelial cells after exposure to ambient ultrafine particles: The role of NADPH oxidase. *Toxicol. Appl. Pharmacol.* 236: 183–193.

Moller, W., Felten, K., Sommerer, K., Scheuch, G., Meyer, G., Meyer, P., Haussinger, K., Kreyling, W.G. 2008. Deposition, retention, and translocation of ultrafine particles from the central airways and lung periphery. *Am. J. Respir. Crit. Care Med.* 177:426–432.

Mundandhara, S.D., Becker, S., Madden, M.C. 2006. Effects of diesel exhaust particles on human alveolar macrophage ability to secrete inflammatory mediators in response to lipopolysaccharide. *Toxicol. In Vitro* 20:614–624.

Mygind, N., Vesterhauge, S. 1978. Aerosol distribution in the nose. *Rhinology* 16:79–88.

Nadadur, S.S., Haykal-Coates, N., Mudipalli, A., Costa, D.L. 2009. Endothelial effects of emission source particles: Acute toxic response gene expression profiles. *Toxicol. In Vitro* 23:67–77.

Nadziejko, C., Fang, K., Bravo, A., Gordon, T. 2007. Susceptibility to pulmonary hypertension in inbred strains of mice exposed to cigarette smoke. *J. Appl. Physiol.* 102:1780–1785.

Nemmar, A. 2003. Possible mechanisms of the cardiovascular effects of inhaled particles: Systemic translocation and prothrombotic effects. *Toxicol. Lett.* 144:s10.

Nemmar, A., Vanbilloen, H., Hoylaerts, M.F., Hoet, P.H.M., Verbruggen, A., Nemery, B. 2001. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. *Am. J. Respir. Crit. Care Med.* 164:1665–1668.

Nemmar, A., Hoet, P.H.M., Vanquickenborne, B., Dinsdale, D., Thomeer, M., Hoylaerts, M.F., Vanbilloen, H., Mortelmans, L., Nemery, B. 2002. Passage of inhaled particles into the blood circulation in humans. *Circulation* 105:411–414.

Nemmar, A., Hoylaerts, M.F., Hoet, P.H.M., Nemery, B. 2003. Pulmonary inflammation and promotion of peripheral vascular thrombosis by particulate pollutants. *Eur. Respir. J.* 22:43s.

Nemmar, A., Hoylaerts, M.F., Hoet, P.H.M., Nemery, B. 2004. Possible mechanisms of the cardiovascular effects of inhaled particles: Systemic translocation and prothrombotic effects. *Toxicol. Lett.* 149:243–253.

Nemmar, A., Al-Maskari, S., Ali, B.H., Al-Amri, I.S. 2007. Cardiovascular and lung inflammatory effects induced by systemically administered diesel exhaust particles in rats. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 292:L664–L670.

Nemmar, A., Dhanasekaran, S., Yasin, J., Ba-Omar, H., Fahim, M.A., Kazzam, E.E., Ali, B.H. 2009. Evaluation of the direct systemic and cardiopulmonary effects of diesel particles in spontaneously hypertensive rats. *Toxicology* 262:50–56.

Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., Kreyling, W., Cox, C. 2002. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J. Toxicol. Environ. Health A* 65:1531–1543.

Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., Cox, C. 2004. Translocation of inhaled ultrafine particles to the brain. *Inhal. Toxicol.* 16:437–445.

Oesterling, E., Chopra, N., Gavalas, V., Arzuaga, X., Lim, E.J., Sultana, R., Butterfield, D.A., Bachas, L., Hennig, B. 2008. Alumina nanoparticles induce expression of endothelial cell adhesion molecules. *Toxicol. Lett.* 178:160–166.

Ovrevik, J., Lag, M., Holme, J.A., Schwarze, P.E., Refsnes, M. 2009. Cytokine and chemokine expression patterns in lung epithelial cells exposed to components characteristic of particulate air pollution. *Toxicology* 259:46–53.

Palecanda, A., Paulauskis, J., Al-Mutairi, E., Imrich, A., Qin, G.Z., Suzuki, H., Kodama, T., Tryggvason, K., Koziel, H., Kobzik, L. 1999. Role of the scavenger receptor MARCO in alveolar macrophage binding of unopsonized environmental particles. *J. Exp. Med.* 189:1497–1506.

Panes, J., Perry, M.A., Anderson, D.C., Manning, A., Leone, B., Cepinskas, G., Rosenbloom, C.L., Miyasaka, M., Kvietys, P.R., Granger, D.N. 1995. Regional differences in constitutive and induced ICAM-1 expression in vivo. *Am. J. Physiol.* 269:H1955–H1964.

Peel, J.L., Metzger, K.B., Klein, M., Flanders, W.D., Mulholland, J.A., Tolbert, P.E. 2007. Ambient air pollution and cardiovascular emergency department visits in potentially sensitive groups. *Am. J. Epidemiol.* 165:625–633.

Peters, A., Liu, E., Verrier, R.L., Schwartz, J., Gold, D.R., Mittleman, M., Baliff, J., Oh, J.A., Allen, G., Monahan, K., Dockery, D.W. 2000. Air pollution and incidence of cardiac arrhythmia. *Epidemiology* 11:11–17.

Pinkerton, K.E., Mercer, R.R., Plopper, C.G., Crapo, J.D. 1992. Distribution of injury and microdosimetry of ozone in the ventilatory unit of the rat. *J. Appl. Physiol.* 73:817–824.

Polichetti, G., Cocco, S., Spinali, A., Trimarco, V., Nunziata, A. 2009. Effects of particulate matter (PM10, PM2.5 and PM1) on the cardiovascular system. *Toxicology* 261:1–8.

Raad, H., Paclet, M.H., Boussetta, T., Kroviarski, Y., Morel, F., Quinn, M.T., Gougerot-Pocidalo, M.A., Dang, P.M.C., El-Benna, J. 2009. Regulation of the phagocyte NADPH oxidase activity: Phosphorylation of gp91(phox)/NOX2 by protein kinase C enhances its diaphorase activity and binding to Rac2, p67(phox), and p47(phox). *FASEB J.* 23:1011–1022.

Ramos, M., Lame, M.W., Segall, H.J., Wilson, D.W. 2007. Monocrotaline pyrrole induces Smad nuclear accumulation and altered signaling expression in human pulmonary arterial endothelial cells. *Vasc. Pharmacol.* 46:439–448.

Rao, S.P., Sikora, L., Hosseinkhani, M.R., Pinkerton, K.E., Sriramarao, P. 2009. Exposure to environmental tobacco smoke induces angiogenesis and leukocyte trafficking in lung microvessels. *Exp. Lung Res.* 35:119–135.

Ren, X., Hsu, P.Y., Dulbecco, F.L., Fleischmann, K.E., Gold, W.M., Redberg, R.F., Schiller, N.B. 2008. Remote second-hand tobacco exposure in flight attendants is associated with systemic but not pulmonary hypertension. *Cardiol. J.* 15:338–343.

Renwick, L.C., Donaldson, K., Clouter, A. 2001. Impairment of alveolar macrophage phagocytosis by ultrafine particles. *Toxicol. Appl. Pharmacol.* 172:119–127.

Renwick, L.C., Brown, D., Clouter, A., Donaldson, K. 2004. Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types. *Occup. Environ. Med.* 61: 442–447.

Riediker, M., Cascio, W.E., Griggs, T.R., Herbst, M.C., Bromberg, P.A., Neas, L., Williams, R.W., Devlin, R.B. 2004. Particulate matter exposure in cars is associated with cardiovascular effects in healthy young men. *Am. J. Respir. Crit. Care Med.* 169:934–940.

Rouse, R.L., Murphy, G., Boudreaux, M.J., Paulsen, D.B., Penn, A.L. 2008. Soot nanoparticles promote biotransformation, oxidative stress, and inflammation in murine lungs. *Am. J. Respir. Cell Mol. Biol.* 39:198–207.

Rueckerl, R., Ibald-Mulli, A., Koenig, W., Schneider, A., Woelke, G., Cyrys, J., Heinrich, J., Marder, V., Frampton, M., Wichmann, H.E., Peters, A. 2006. Air pollution and markers of inflammation and coagulation in patients with coronary heart disease. *Am. J. Respir. Crit. Care Med.* 173:432–441.

Ryan, U.S. 1987. Activation of endothelial cells. *Annals of the New York Academy of Sciences* 516:22–38.

Samet, J.M., Rappold, A., Graff, D., Cascio, W.E., Berntsen, J.H., Huang, Y.-C.T., Herbst, M., Bassett, M., Montilla, T., Hazucha, M.J., Bromberg, P.A., Devlin, R.B. 2009. Concentrated ambient ultrafine particle exposure induces cardiac changes in young healthy volunteers. *Am. J. Respir. Crit. Care Med.* 179:1034–1042.

Schleimer, R.P., Kato, A., Kern, R., Kuperman, D., Avila, P.C. 2007. Epithelium: At the interface of innate and adaptive immune responses. *J. Allergy Clin. Immunol.* 120:1279–1284.

Schwab, A.J., Salamand, A., Merhi, Y., Simard, A., Dupuis, J. 2003. Kinetic analysis of pulmonary neutrophil retention in vivo using the multiple-indicator-dilution technique. *J. Appl. Physiol.* 95: 279–291.

Seaton, A., Macnee, W., Donalson, K., Godden, D. 1995. Particulate air pollution and acute health effects. *Lancet* 345:176–178.

Semaeva, E., Tenstad, O., Bletsa, A., Gjerde, E.-A.B., Wiig, H. 2008. Isolation of rat trachea interstitial fluid and demonstration of local cytokine production in lipopolysaccharide-induced systemic inflammation. *J. Appl. Physiol.* 104:809–820.

Semberova, J., Holada, K., Simakova, O., Gelderman-Fuhrmann, M.P., Simak, J. 2008. Carbon nanotubes activate platelets by facilitating extracellular Ca2+ influx. *Blood* 112:365–366.

Semmler-Behnke, M., Takenaka, S., Fertsch, S., Wenk, A., Seitz, J., Mayer, P., Oberdorster, G., Kreyling, W.G. 2007. Efficient elimination of inhaled nanoparticles from the alveolar region: Evidence for interstitial uptake and subsequent reentrainment onto airway epithelium. *Environ. Health Perspect.* 115:728–733.

Singal, M., Finkelstein, J.N. 2005. Amorphous silica particles promote inflammatory gene expression through the redox sensitive transcription factor, AP-1, in alveolar epithelial cells. *Exp. Lung Res.* 31:581–597.

Soberanes, S., Urich, D., Baker, C.M., Burgess, Z., Chiarella, S.E., Bell, E.L., Ghio, A.J., De Vizcaya-Ruiz, A., Liu, J., Ridge, K.M., Kamp, D.W., Chandel, N.S., Schumacker, P.T., Mutlu, G.M., Budinger, G.R.S. 2009. Mitochondrial complex III-generated oxidants activate ASK1 and JNK to induce alveolar epithelial cell death following exposure to particulate matter air pollution. *J. Biol. Chem.* 284: 2176–2186.

Squadrito, G.L., Cueto, R., Dellinger, B., Pryor, W.A. 2001. Quinoid redox cycling as a mechanism for sustained free radical generation by inhaled airborne particulate matter. *Free Radic. Biol. Med.* 31:1132–1138.

Stevens, T., Phan, S., Frid, M.G., Alvarez, D., Herzog, E., Stenmark, K.R. 2008. Lung vascular cell heterogeneity: Endothelium, smooth muscle, and fibroblasts. *Proc. Am. Thorac. Soc.* 5:783–791.

Stoll, L.L., Denning, G.M., Weintraub, N.L. 2004. Potential role of endotoxin as a proinflammatory mediator of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 24:2227–2236.

Sun, Q., Wang, A., Jin, X., Aguinaldo, J.-G.S., Duquaine, D., Natanzon, A., Brook, R.D., Fayad, Z.A., Fuster, V., Chen, L.C., Rajagopalan, S. 2005a. Chronic airborne pollution potentiates atherosclerosis in hyperlipidemic mice. *Circulation* 112:U348.

Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.-G.S., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C., Rajagopalan, S. 2005b. Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. *JAMA* 294:3003–3010.

Sun, Q., Yue, P., Kirk, R., Wang, A., Moatti, D., Jin, X., Lu, B., Schecter, A., Lippmann, M., Gordon, T., Chen, L., Rajagopalan, S. 2008. Ambient air particulate matter exposure and tissue factor expression in atherosclerosis. *Inhal. Toxicol.* 20:127–137.

Suwa, T., Hogg, J.C., Quinlan, K.B., Ohgami, A., Vincent, R., van Eeden, S.F. 2002. Particulate air pollution induces progression of atherosclerosis. *J. Am. Coll. Cardiol.* 39:935–942.

Takano, H., Yanagisawa, R., Ichinose, T., Sadakane, K., Yoshino, S., Yoshikawa, T., Morita, M. 2002. Diesel exhaust particles enhance lung injury related to bacterial endotoxin through expression of proinflammatory cytokines, chemokines, and intercellular adhesion molecule-1. *Am. J. Respir. Crit. Care Med.* 165:1329–1335.

Tamagawa, E., Bai, N., Morimoto, K., Gray, C., Mui, T., Yatera, K., Zhang, X., Xing, L., Li, Y., Laher, I., Sin, D.D., Man, S.F.P., van Eeden, S.F. 2008. Particulate matter exposure induces persistent lung inflammation and endothelial dysfunction. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 295:L79–L85.

Terada, L.S. 2006. Specificity in reactive oxidant signaling: Think globally, act locally. *J. Cell Biol.* 174:615–623.

Terry, R.W., Kwee, L., Levine, J.F., Labow, M.A. 1993. Cytokine induction of an alternatively spliced murine vascular cell adhesion molecule (VCAM) mRNA encoding a glycosylphosphatidylinositol-anchored VCAM protein. *Proc. Natl. Acad. Sci. USA* 90:5919–5923.

Thomson, E., Goegan, P., Kumarathasan, P., Vincent, R. 2004. Air pollutants increase gene expression of the vasoconstrictor endothelin-1 in the lungs. *Biochim. Biophys. Acta* 1689:75–82.

Thomson, E., Kumarathasan, P., Goegan, P., Aubin, R.A., Vincent, R. 2005. Differential regulation of the lung endothelin system by urban particulate matter and ozone. *Toxicol. Sci.* 88:103–113.

Thomson, E., Kumarathasan, P., Vincent, R. 2006. Pulmonary expression of preproET-1 and preproET-3 mRNAs is altered reciprocally in rats after inhalation of air pollutants. *Exp. Biol. Med. (Maywood)* 231:979–984.

Tong, H., McGee, J.K., Saxena, R.K., Kodavanti, U.P., Devlin, R.B., Gilmour, M.I. 2009. Influence of acid functionalization on the cardiopulmonary toxicity of carbon nanotubes and carbon black particles in mice. *Toxicol. Appl. Pharmacol.* 239:224–232.

Tornqvist, H., Mills, N.L., Gonzalez, M., Miller, M.R., Robinson, S.D., Megson, I.L., MacNee, W., Donaldson, K., Soderberg, S., Newby, D.E., Sandstrom, T., Blomberg, A. 2007. Persistent endothelial dysfunction in humans after diesel exhaust inhalation. *Am. J. Respir. Crit. Care Med.* 176:395–400.

Upham, J.W., Stumbles, P.A. 2003. Why are dendritic cells important in allergic diseases of the respiratory tract? *Pharmacol. Ther.* 100:75–87.

Urch, B., Silverman, F., Corey, P., Brook, J.R., Lukic, K.Z., Rajagopalan, S., Brook, R.D. 2005. Acute blood pressure responses in healthy adults during controlled air pollution exposures. *Environ. Health Perspect.* 113:1052–1055.

Urich, D., Soberanes, S., Burgess, Z., Chiarella, S.E., Ghio, A.J., Ridge, K.M., Kamp, D.W., Chandel, N.S., Mutlu, G.M., Budinger, G.R.S. 2009. Proapoptotic Noxa is required for particulate matter-induced cell death and lung inflammation. *FASEB J.* 23:2055–2064.

Ushio-Fukai, M. 2007. VEGF signaling through NADPH oxidase-derived ROS. *Antioxidants & Redox Signaling* 9:731–739.

Ushio-Fukai, M. 2009. Compartmentalization of redox signaling through NADPH oxidase-derived ROS. *Antioxidants & Redox Signaling* 11:1289–1299.

Veranth, J.M., Moss, T.A., Chow, J.C., Labban, R., Nichols, W.K., Walton, J.C., Watson, J.G., Yost, G.S. 2006. Correlation of in vitro cytokine responses with the chemical composition of soil-derived particulate matter. *Environ. Health Perspect.* 114:341–349.

Vermylen, J., Nemmar, A., Nemery, B., Hoylaerts, M.F. 2005. Ambient air pollution and acute myocardial infarction. *J. Thromb. Haemost.* 3:1955–1961.

Vidrio, E., Jung, H., Anastasio, C. 2008. Generation of hydroxyl radicals from dissolved transition metals in surrogate lung fluid solutions. *Atmos. Environ.* 42:4369–4379.

Watkinson, W.P., Campen, M.J., Costa, D.L. 1998. Cardiac arrhythmia induction after exposure to residual oil fly ash particles in a rodent model of pulmonary hypertension. *Toxicol. Sci.* 41:209–216.

West, J.B., Breen, E.C., Mathieu-Costello, O. 1998. Strength, failure, and remodeling of the pulmonary blood-gas barrier. In: Weir, E.K., Reeves, J.T. (eds). *Pulmonary Edema*. New York: Futura Publishing Company, pp. 171–182.

Wichers, L.B., Nolan, J.P., Winsett, D.W., Ledbetter, A.D., Kodavanti, U.P., Schladweiler, M.C.J., Costa, D.L., Watkinson, W.P. 2004. Effects of instilled combustion-derived particles in spontaneously hypertensive rats. Part I: Cardiovascular responses. *Inhal. Toxicol.* 16:391–405.

Wilson, D.W., Segall, H.J., Pan, L.C.W., Dunston, S.K. 1989. Progressive inflammatory and structural changes in the pulmonary vasculature of monocrotaline-treated rats. *Microvasc. Res.* 38:57–80.

Wu, R.-F., Ma, Z., Liu, Z., Terada, L.S. 2010. Nox4-derived H_2O_2 mediates endoplasmic reticulum signaling through local Ras activation. *Molecular and Cellular Biology* 30:3553–3568.

Yatera, K., Hsieh, J., Hogg, J.C., Tranfield, E., Suzuki, H., Shih, C.-H., Behzad, A.R., Vincent, R., van Eeden, S.F. 2008. Particulate matter air pollution exposure promotes recruitment of monocytes into atherosclerotic plaques. *Am. J. Physiol. Heart Circ. Physiol.* 294:H944–H953.

Yin, J., Kuebler, W.M. 2009. Mechanotransduction by TRP channels: General concepts and specific role in the vasculature. *Cell Biochem. Biophys.* 56:1–18.

Zhao, Y.T., Natarajan, V. 2009. Lysophosphatidic acid signaling in airway epithelium: Role in airway inflammation and remodeling. *Cell. Signal.* 21:367–377.

Zhou, H.W., Kobzik, L. 2007. Effect of concentrated ambient particles on macrophage phagocytosis and killing of *Streptococcus pneumoniae*. *Am. J. Respir. Cell Mol. Biol.* 36:460–465.

CARDIOVASCULAR EFFECTS OF INHALED ULTRAFINE AND NANOSIZED PARTICLES

Edited by

FLEMMING R. CASSEE
National Institute for Public Health and the Environment
Bilthoven, The Netherlands

NICHOLAS L. MILLS
University of Edinburgh
Edinburgh, United Kingdom

DAVID NEWBY
University of Edinburgh
Edinburgh, United Kingdom

WILEY

A JOHN WILEY & SONS, INC., PUBLICATION

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

Library of Congress Cataloging-in-Publication Data is available.

ISBN 978-0-470-43353-9

Printed in Singapore

10 9 8 7 6 5 4 3 2 1

PART IV

PARTICLES AND CARDIOVASCULAR DISEASE: MECHANISMS B. VASCULAR DYSFUNCTION