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Serial Review: Role of Reactive Oxygen and Nitrogen Species (ROS/RNS) in Lung Injury and Diseases
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OXIDATIVE STRESS AND CALCIUM SIGNALING IN THE ADVERSE EFFECTS OF ENVIRONMENTAL PARTICLES (PM_{10})

KEN DONALDSON,* VICKI STONE,*† PAUL J. A. BORM,‡ LUIS A. JIMENEZ,* PETER S. GILMOUR,* ROEL P. F. SCHINS,‡ AD M. KNAAPEN,‡ IRFAN RAHMAN,* STEPHEN P. FAUX,*§ DAVID M. BROWN,† and WILLIAM MACNEE*

*Colt/ELEGI Laboratories, Centre for Inflammation Research, The University of Edinburgh Medical School, Edinburgh, UK; †School of Life Sciences, Napier University, Edinburgh, UK; ‡Particle Research Core, Institute for Environmental (IUF) at the Heinrich-Heine-University, Düsseldorf, Germany; and §Institute of Occupational Medicine, Edinburgh, UK

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Abstract—This review focuses on the potential role that oxidative stress plays in the adverse effects of PM_{10}. The central hypothesis is that the ability of PM_{10} to cause oxidative stress underlies the association between increased exposure to PM_{10} and both exacerbations of lung disease and lung cancer. Pulmonary inflammation may also underlie the cardiovascular effects seen following increased PM_{10}, although the mechanisms of the cardiovascular effects of PM_{10} are not well understood. PM_{10} is a complex mix of various particle types and several of the components of PM_{10} are likely to be involved in the induction of oxidative stress. The most likely of these are transition metals, ultrafine particle surfaces, and organic compounds. In support of this hypothesis, oxidative stress arising from PM_{10} has been shown to activate a number of redox-responsive signaling pathways in lung target cells. These pathways are involved in expression of genes that play a role in responses relevant to inflammation and pathological change, including MAPKs, NF-κB, AP-1, and histone acetylation. Oxidative stress from particles is also likely to play an important role in the carcinogenic effects associated with PM_{10} and hydroxyl radicals from PM_{10} cause DNA damage in vitro. © 2003 Elsevier Inc.

Keywords—Free radicals, Air pollution, PM_{10}, Particles, Inflammation

THE ADVERSE EFFECTS OF PM_{10}

Effects of PM_{10} on the lung and the cardiovascular system

Ambient particles are quantified by the PM_{10} convention, defined as a convention that measures the mass of ambient airborne particles that range from about 15 μm aerodynamic diameter down to ultrafine particles, with 50% efficiency for particles with an aerodynamic diameter of 10 μm [1]. Although other conventions are important, e.g., $PM_{2.5}$, most of the epidemiological and toxicology data are derived from PM_{10}, and so we use the term PM_{10} as convenient shorthand for ambient particles as measured globally unless another convention is explicitly considered, e.g., 2.5.

Numerous epidemiological studies have described an association between increased levels of PM_{10} and exacerbations of airways disease in patients with Chronic Obstructive Pulmonary Disease (COPD) and asthma [2]. In addition, time-series studies show that there is also an increase in cardiovascular deaths and hospital admissions associated with increases in the levels of PM_{10} [3,4]. There is a relationship between chronic exposure to high PM_{10} levels and cancer [5,6]. This review addresses the potential role of oxidative stress in leading to the adverse effects of PM_{10} and this is summarized in Fig. 1.

This article is part of a series of reviews on "Role of Reactive Oxygen and Nitrogen Species (ROS/RNS) in Lung Injury and Diseases." The full list of papers may be found on the homepage of the journal.

Address correspondence to: Prof. Kenneth Donaldson, Colt/ELEGI Laboratories, Centre for Inflammation Research, The University of Edinburgh Medical School, Edinburgh EH 8 9AG, Scotland; Tel: 0131 651 1437; Fax: 0131 651 1558; E-Mail: ken.donaldson@ed.ac.uk.

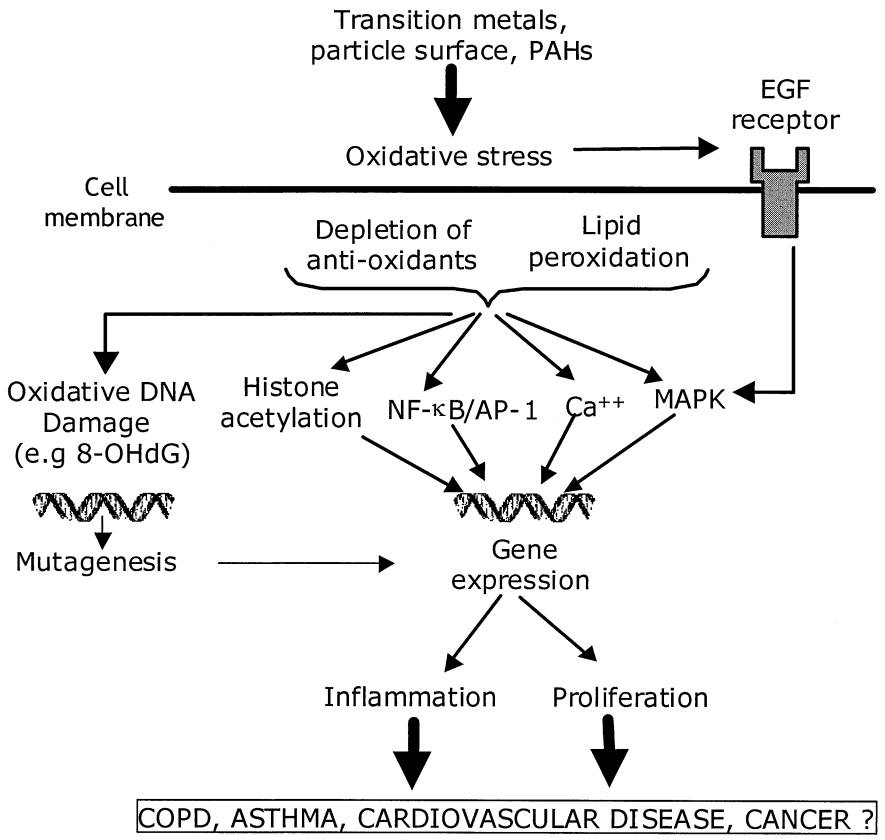

Fig. 1. Simplified diagram of hypothetical oxidative stress-mediated effects of PM_{10} leading to adverse health effects. The diagram brings together the mechanisms and pathways described in the review.

Oxidative stress and the adverse effects of PM_{10}

Inflammation is a mechanism in the adverse effects of all pathogenic particles [7] and is likely to be an important driver of the adverse effects of PM_{10}. PM_{10} has been shown to have the ability to cause inflammation in humans exposed to concentrated airborne ambient particles [8] and instilled PM_{10} [9], and in animal models, following instillation [10]. Both asthma [11] and COPD [12] are diseases characterized by inflamed airways. Exacerbations of asthma and COPD feature increased severity of the inflammatory response, which could be triggered by increased particle-derived oxidative stress and inflammation in the lungs [13,14]. PM_{10}-mediated inflammation could also play a role in precipitating cardiovascular deaths and hospital admissions through an effect on coronary artery disease, which has an important inflammatory component [15]. Free radicals and oxidative stress have been extensively implicated in the inflammatory effects of PM_{10}. However, the same species may also contribute to the observed epidemiological association between lung cancer and exposure to PM via genotoxic effects.

Heterogeneity in the toxic potency of the components of PM_{10}

The components of PM_{10} represent a complex mixture that can be divided on toxicological grounds into:

1. Primary particles
2. Secondary particles
3. Organics, e.g., PAHs
4. Crustal minerals
5. Biologically derived material

Primary particles, especially those from traffic sources, are considered amongst the most harmful components of PM_{10}. These particles are formed by combustion processes such as petrol burning in cars and have a small primary particle size, often in the ultrafine range ($<$100 nm diameter); this means that they have a large surface area per unit mass on which free radical and catalytic reactions can occur. The combustion process also means that they are likely to have associated metals and organic molecules such as Polycuclic Aromatic Hydrocarbons (PAHs). The presence of transition metals in PM_{10} is a dominant hy-

pothesis for PM_{10} toxicity [16] via the generation of free radicals by Fenton chemistry [17], and PAHs are also able to contribute to increased ability of PM_{10} to cause inflammation [18,19]. Further support for the hypothesis that the primary component of PM_{10} is important in mediating the adverse health effects has come from studies where human subjects exposed to low levels of diesel soot rapidly develop inflammatory responses in their lungs [20]. In vitro studies with epithelial cells exposed to diesel soot reveal that proinflammatory genes are switched on and that these effects are driven by free radical/oxidative stress mechanisms [21–23].

The secondary components of PM_{10} form as a result of atmospheric chemistry involving sulphur dioxide (SO_2), oxides of nitrogen (Nox), ammonia, and sunlight [16]. These are typified by the ammonium sulphates, nitrates, and chlorides, and very likely have little toxicological significance.

The crustal mineral component is usually coarse and contains wind-blown and re-entrained dust that is rich in minerals such as aluminum silicate clays and soil particles, depending on the situation. These crustal elements are not, in general, considered to be potent in causing adverse effects.

Small particles of biologically derived materials such as plant parts, some fungal spores, and bacteria are also within the PM_{10} convention. These biological components are not considered, in general, to be very likely to contribute to pathogenicity of PM_{10}, with the key exception of endotoxin, an element of gram-negative bacterial cell walls that is highly potent in causing inflammation. Endotoxin has been reported to mediate some of the proinflammatory effects of some PM samples [24,25], although not all samples [26].

The oxidative stress hypothesis for the adverse effects of PM_{10}

Oxidative stress is a central hypothetical mechanism for the adverse effects of PM_{10} [7] via metal-generated free radicals [27,28], ultrafine particle surfaces [29] and organic components [30,31]. Evidence that particle-derived oxidative stress may play a role in initiating and prolonging inflammation, and in causing genotoxic effects, is described below. Since several of the components of PM_{10} can generate oxidative stress, there is potential for additive or synergistic interaction between the components [32]. We have recently provided evidence that there can be synergistic interactions between transition metals and ultrafine particles in causing oxidative stress and lung inflammation [33].

ROLES OF OXIDATIVE STRESS IN SIGNALING AND INFLAMMATION

Oxidative stress and signaling

The induction of inflammatory mediators can be regulated by the activation of redox-sensitive transcription factors activator protein 1 (AP-1) and and nuclear factor kappa B (NF-κB) stimulated in response to reactive oxygen species (ROS). Cellular redox status, particularly intracellular thiol status, can be directly involved in the activation of AP-1 and NF-κB [34–36], signal transduction, and gene expression involved in pathophysiological events [37]. Both environmental and inflammatory cell-derived ROS can lead to increases in intracellular calcium, the activation and phosphorylation of the mitogen activated protein kinase (MAPK) family, including extracellular signal regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38 kinase, and PI-3K/Akt via sensitive cysteine-rich domains and the sphingomyelinase-ceramide pathway, leading to increased gene transcription [38–40]. Activation of members of the MAPK family leads to the transactivation of transcription factors such as c-Jun, activating factor-2 (ATF2), and cyclic AMP response element binding proteins (CREB)-binding protein (CBP) [37,40–42]. This eventually results in chromatin remodeling and expression of genes regulating a battery of genes involved in inflammation, apoptosis, proliferation, transformation, and differentiation. These pathways are discussed in detail below in relation to the effects of PM_{10}.

Cellular ROS

Chronic inflammatory lung diseases are characterized by activation of epithelial cells and resident macrophages, and the recruitment and activation of neutrophils, eosinophils, monocytes, and lymphocytes. The activation of macrophages, neutrophils, and eosinophils generates ROS, but this is not dealt with here. This review focuses on the role of the particles themselves in generating oxidative stress, although this will clearly be amplified by ROS released by lung cells.

OXIDATIVE STRESS AND THE ADVERSE EFFECTS OF PM_{10}

Calcium

One mechanism by which oxidative stress from PM_{10} may induce the expression of proinflammatory mediators is via stimulation of intracellular signaling pathways that employ calcium [43]. Intracellular calcium acts as a key signaling mechanism which, through interaction with a number of proteins such as calmodulin and enzymes such as protein kinases, is able to regulate the activation

of a number of key transcription factors. Such transcription factors include nuclear factor of activated T cells (NFAT) and NFκB [44,45].

Oxidants have been reported to cause a rapid increase in cytosolic calcium concentration in a wide variety of cell types, including hepatocytes and muscle cells [46–48] There are a number of hypothetical mechanisms by which oxidative stress could stimulate or modulate an intracellular calcium event. For example, the major intracellular calcium store, which is located within the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR) [49], can be released via stimulation of G receptors on the cell surface and initiation of an intracellular signaling cascade involving inositol 1,4,5-triphoshate (IP_3). In order to cause calcium release from the ER, IP_3 interacts with an IP_3 receptor on the surface of the ER which, once stimulated, acts as a calcium channel allowing calcium to empty from the ER into the cytoplasm. Both production of IP_3 [50] and release of calcium from the ER store [47] have been suggested to occur on exposure of cells to oxidative stress. Emptying of the ER/SR calcium store can also be induced by inhibiting the ER/SR Ca^{2+} adenosine triphosphatase (ATPase), which is responsible for pumping calcium into these stores. Again, exposure to oxidants can inhibit these pumps due to oxidation of sulphydryl groups or attack of the adenosine triphosphate (ATP) binding site [51,52]. The effect of reactive oxygen species and free radicals on the IP_3 receptor has yet to be elucidated, but could also provide a route for interaction between oxidants and calcium signaling.

An alternative source of cytosolic calcium is the extracellular environment from where calcium may enter the cell via a variety of channels in the plasma membrane [43]. Again, oxidative stress has been shown to impact on this pathway [47] in that the activity of these channels is altered by reactive oxygen species [53].

Calcium signaling events and oxidants have been demonstrated to be important in the induction of transcription factor activation and cytokine gene expression by macrophages [54] treated with ultrafine particles, an important component of PM_{10}. In these experiments, exposure of both a human monocytic cell line [55,56] and rat alveolar macrophages [57] to either ultrafine carbon or polystyrene particles stimulated a rapid increase in cytosolic calcium concentration, which was not associated with cell death. In addition to increasing the resting cytosolic calcium concentration, ultrafine particles also induced a significant increase in the cytosolic calcium response to stimulation with thapsigargin (50 nM). Thapsigargin [58] is an agonist frequently used to stimulate a rise in cytosolic calcium by inhibiting the ER Ca^{2+} ATPase. Inhibition of the ER Ca^{2+} ATPase induces emptying of the ER store, which subsequently stimulates opening of calcium channels in the plasma membrane and an influx of extracellular calcium. Exposure of macrophages to ultrafine particles at concentrations of up to 66 μg/ml for as little as 10 min induced a large increase in the response to thapsigargin, which could be blocked by the calcium channel blocker verapamil (100 μM), suggesting that the ultrafine particles enhanced opening of the plasma membrane calcium channels [55,59]. Further evidence to support this assumption was gained by repeating the experiment in the absence of extracellular calcium, which resulted in a reversal of the calcium current across the plasma membrane. This was further supported by conducting the experiment in the presence of extracellular manganese, which enters the cell via the calcium channels and combined with the calcium sensitive dye, and demonstrating quenching of fura-2. In the absence of extracellular calcium the ultrafine particles did not significantly alter the amplitude of the calcium release from the ER induced by thapsigargin, again supporting the conclusion that the additional cytosolic calcium was not derived directly from the ER but was extracellular in origin.

Ultrafine particles are known to generate reactive oxygen species and cause oxidative stress [29,56]. The ability of oxidative stress to enhance both IP_3 production and calcium signaling events has been demonstrated in various cell types, including endothelial cells [60] and macrophages [50]. For this reason, the role of particle-induced oxidative stress in the calcium response was investigated using the antioxidants nacystelin and mannitol, both of which diminished the ultrafine carbon black-enhanced thapsigargin response [29,56]. However, this inhibition was not absolute, suggesting that the effect may not be solely due to oxidative stress. The relationship between intracellular calcium and oxidative stress may be complex in that oxidative stress may initiate the calcium signal, which then activates further cellular production of reactive oxygen species such as superoxide anion radicals [61] or nitric oxide [62]. These reactive oxygen species may then further enhance the calcium signal [43] and consequently amplify any downstream events such as transcription factor activation and cytokine gene expression.

In accordance with this data and hypothesis, treatment of rat alveolar macrophages [54,63] and human-derived monocyte-derived macrophages [64] with ultrafine carbon black for 4 h stimulated a significant increase in the production of both tumor necrosis factor alpha (TNFα) protein and messenger ribonucleic acid (mRNA). The production of TNFα protein was mediated by a pathway involving calcium, since this response was completely inhibited by both the calcium channel blocker verapamil (Fig. 2) and an intracellular calcium chelator (BAPTA-AM). Furthermore, the addition of antioxidants such as

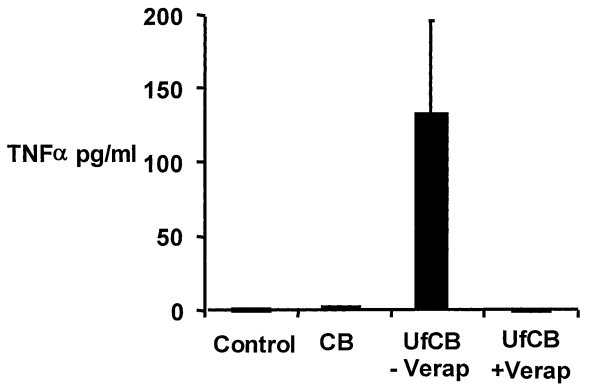

Fig. 2. TNFα release by Monomac6 macrophage-like cells following treatment for 4 h with fine carbon back (CB: average diameter approximately 250 nm) or ultrafine carbon black (UfCB: average diameter approximately 16 nm). UfCB treatments were either with (+ Verap) or without (− Verap) co-treatment with the calcium channel-blocker Verapamil. TNFα release is stimulated by UfCB but not CB and a clear role for calcium is shown by the abolition of TNFα release in the presence of Verapamil.

nacystelin and trollox both caused a partial inhibition of the ultrafine particle-induced TNFα expression.

In addition to stimulating TNFα production, the ultrafine particles also stimulated an increase in NFκB nuclear translocation in the human monocytes as determined by immunohistochemistry of the p65 subunit [63]. The role of calcium in controlling NFκB nuclear translocation in response to ultrafine particle exposure appears to be similar to its role in regulating TNFα expression, since NFκB nuclear translocation was inhibited completely by the calcium antagonists verapamil and BAPTA-AM, and partially by the antioxidants nacystelin and trollox. The involvement of calcium in the activation of NFκB in macrophages has also been demonstrated in rat Kupffer cells co-cultured with hepatoma cells [65].

Taken together, these data indicate that ultrafine particles stimulate both oxidative stress and intracellular calcium signaling events in macrophages. The calcium signals are in part initiated, and may even be enhanced, in response to particle-induced oxidative stress. Furthermore, the calcium signals stimulate nuclear translocation of NF-κB and expression of TNFα protein, which would be anticipated to induce or exacerbate inflammation in vivo. The role of calcium in other cellular responses in which oxidative stress has been implicated is yet to be determined.

Activation of the mitogen-activated protein kinase cascades

The mitogen-activated protein kinases (MAPK) are a super family of protein kinases that are ubiquitously expressed in mammalian cells. They are intimately involved in signal transduction through their ability to phosphorylate a number of proteins and transcription factors, and are reviewed elsewhere [66,67]. They have been implicated in the pathogenic effects of a range of other pathogenic particles.

Asbestos. Studies have shown that MAPKs are activated after exposure of rat pleural mesothelial (RPM) cells to asbestos fibers at concentrations inducing increased expression of the protooncogenes c-fos and c-jun [68]. A range of pathogenic fibers, including asbestos, phosphorylate, and activate extracellularly regulated kinase (ERK) after autophosphorylation of the epidermal growth factor-receptor (EGF-R), whereas chemically identical nonfibrous counterparts of asbestos did not. Exposure of RPM cells to asbestos depleted their glutathione [69], and pretreatment with an antioxidant abrogated asbestos-induced activation of ERKs and apoptosis induced by ERK activation [70]. Swain and Faux [71] confirmed a role for oxidative stress by demonstrating that the increased phosphorylation of p38 MAPK in both RPM cells and a human mesothelial cell line, MET5A, following stimulation with crocidolite asbestos, could be modulated by pretreatment with NAC and α-tocopherol (vitamin E), an inhibitor of lipid peroxidation. When RPM cells were preincubated with PKI166, an inhibitor of the EGF-R, p38 activation was not inhibited, indicating that, unlike ERK, p38 is not activated following phosphorylation of a growth factor receptor. p38 was shown to be involved in activator protein-1 (AP-1) DNA binding as SB203580, a specific p38 inhibitor, reduced AP-1 DNA binding activity [71].

Silica. Studies by Mossman and co-workers [72] showed that the fibrogenic and carcinogenic particle, crystalline silica, induced the activation of the c-Jun N-terminal kinase (JNK) pathway in a murine alveolar type II epithelial cell line in a dose- and time-dependent manner with maximal induction at 8 h. These effects were shown to be linked to downstream AP-1-dependent gene expression. Other studies with freshly fractured silica showed that increases in AP-1 were dependent on both the ERK and p38 pathways [73].

PM_{10}. There have been a limited number of studies into the effects of PM_{10} on the MAPK pathway. Increases in JNK activity in alveolar epithelial cells, accompanied by increases in phosphorylated cJun and transcriptional activation of elements of the AP-1 complex, have been demonstrated [74]. These changes were accompanied by elevation in incorporation of 5'-bromodeoxyuridine, a marker of DNA synthesis and cell proliferation. Investigations with Utah Valley PM showed increases in ERK1/2 phosphorylation following activation of the EGF-R [75]. Treatment of alveolar macrophages with residual oil fly ash (ROFA) particulates demonstrated

that JNK and p38, but not ERK, were activated [76]. Diesel exhaust particulates (DEP) activated p38 MAPK and induced IL-8 production in human bronchial epithelial cells [77]. These effects were due to oxidative stress as NAC could attenuate p38 activation and IL-8 production. Organic extracts of DEP activate ERKs and p38 [78,79], leading to IL-8 release, and these effects can be attenuated by incubation with NAC [79]. A number of studies into particulate matter have suggested that transition metals are important in causing oxidative stress and lung injury [23]. Metals, such as arsenic, zinc, and vanadium, activate ERK1/2 following phosphorylation of the EGF-R in human airway epithelial cells [80].

These studies confirm that the MAPKs are an important target for PM_{10} via oxidative stress mechanisms. Further studies are required with the components of PM_{10} to determine which of these are most important in the effects discussed above. There also needs to be elucidation of the role that downstream physiological effects of MAPK activation, such as proliferation, differentiation, and cell death of apoptosis, play in the known adverse effects of PM_{10}, such as exacerbations of airways disease and cardiovascular complications.

NF-κB pathway

NF-κB. Nuclear factor-kappa b (NF-κB) is a collective term referring to ubiquitously expressed redox-sensitive transcription factors belonging to the Rel family of proteins, and are reviewed elsewhere [81,82]. NF-κB is activated in response to various oxidative stresses and is fundamentally involved in inflammation, apoptosis, and proliferation.

NF-κB and PM_{10}. The NF-κB signaling pathway has been shown to be stimulated in response to a range of pathogenic particles (reviewed in [83]). DEP, an important constituent of air pollution, and its organic extracts induce NF-κB activity and IκB degradation in human bronchial epithelial (16HBE) cells, and these responses are abrogated by the hydroxyl scavenger dimethyl thiourea [84,85]. Similarly, benzene-extracted components of DEP and benzo[a]pyrene induced NF-κB activity and expression and release of a number of proinflammatory genes in BEAS-2B cells [79].

Several investigators have shown that the biological effects of ambient particulates on intracellular signaling and induction of proinflammatory mediators from lung cells can be partially attributed to the presence of transition metals. Kennedy and co-workers [86] showed PM_{10} collected from Provo, Utah enhanced NF-κB DNA-binding and interleukin-6 (IL-6) release from the bronchial cell line BEAS, an effect that was mimicked with copper. In addition, studies conducted by the same group using residual oil fly ash (ROFA), showed a time and dose-dependent increase in IL-6 messenger RNA (mRNA) that was preceded by NF-κB activation [87]. ROFA particles induced transcriptional activation of an IL-6 promoter containing κB binding sites, a response that was attenuated using the metal chelator deferoxamine and NAC. Vanadium, a component of ROFA, triggered both epidermal growth factor receptor (EGFR) activation and Ras-dependent activation of NF-κB [80,88], and vanadium-induced NF-κB activity and IκB degradation were ameliorated by either pretreatment of BEAS-2B with PD-153035, an inhibitor of EGFR activity, or overexpression of the dominant negative Ras (N17); this suggests that vanadium stimulates NF-κB through cross-talk with the EGFR signaling pathway at the level of Ras.

The importance of iron in PM_{10}-induced NF-κB has also been demonstrated. Jimenez and co-workers [23] showed that PM_{10} collected in the United Kingdom induced nuclear translocation, DNA-binding, and transcriptional activation of NF-κB, which occurred in the absence of IκB degradation in human alveolar epithelial (A549) cells (Fig. 3A). Treatment of PM_{10} with both deferoxamine and ferrozine, transition metal chelators with high affinities for Fe2+ and Fe3+, respectively, completely inhibited NF-κB activation (Fig. 3B). Furthermore, exposure of A549 cells to soluble fractions from phosphate-buffered saline-treated PM_{10} activated NF-κB, indicating that soluble components such as metals or organic materials present on the surface of PM_{10} are themselves capable of activating NF-κB. Mossman and co-workers [89] showed that inhalation of $PM_{2.5}$ in mice upregulated several NF-κB-mediated genes, including TNF-α, IL-6, and transforming growth factor-β. $PM_{2.5}$ also enhanced transcriptional activation of a NF-κB-luciferase reporter in murine alveolar type II epithelial cells, which was attenuated in the presence of catalase. In the same study, ultrafine carbon black, a surrogate for the ultrafine fraction of PM_{10} and known to induce oxidative stress in lung cells [56], stimulated transcriptional activation of NF-κB. Taken together, the results imply that transition metals and ultrafine particles are active constituents of particulate air pollution that stimulate intracellular signaling pathways, leading to NF-κB activation and enhanced cytokine transcription in lung cells through oxidant-mediated mechanisms.

NF-κB and susceptibility. The presence of the adenovirus regulatory gene E1A has been suggested to be a possible factor in susceptibility to inflammation caused by cigarette smoke. The E1A gene has been found to be present at a higher frequency in the lungs of COPD patients than similar smokers without COPD [90]. The presence of E1A also enhances the inflammatory re-

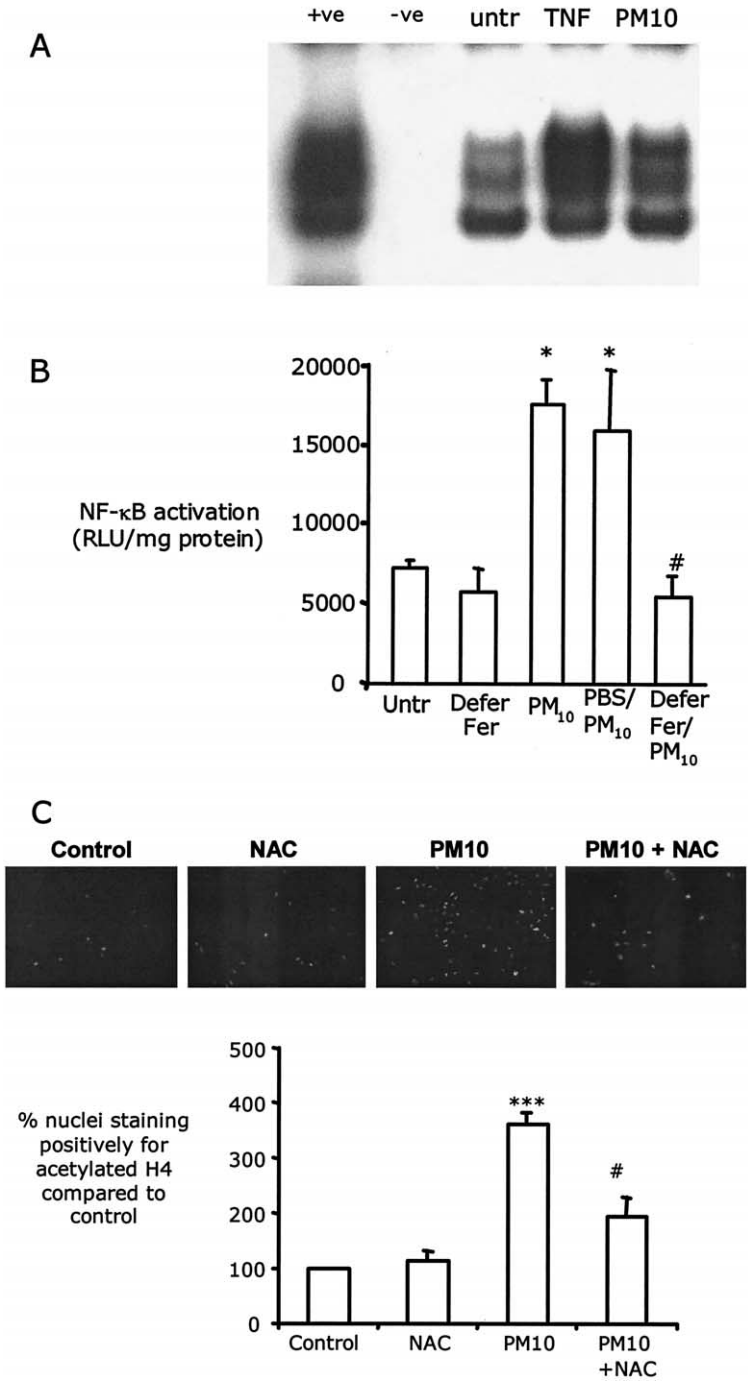

Fig. 3. (A) PM_{10}-enhanced DNA-binding of the transcription factor, NF-κB. Nuclear extracts from A549 cells exposed to PM_{10} or TNF-α for 2 h were analyzed by electrophoretic mobility shift assay. HeLa extract and binding buffer were used as positive (+ve) and negative (−ve) controls for the gel shift reaction. (B) Attenuation of PM_{10}-induced transcriptional activation of NF-κB by transition metal chelation. A549 cells stably transfected with a NF-κB-luciferase reporter construct were exposed for 18 h to PM_{10} or PM_{10} treated with either PBS or the transition metal chelators deferoxamine + ferrozine. Cell lysates were prepared and luciferase activity was determined by luminescence. Results are mean + SEM relative luciferase units per milligram of protein of three separate experiments (RLU/mg protein). * $p < .05$ compared to Neg; # $p < .05$ compared to PM_{10}. Defer Fer = Deferoxamine+Ferrozine; PBS = phosphate-buffered saline; Untr = untreated A549 cells. Adapted from [23]. (C) A549 cells were treated with PM_{10} (100 μg/ml) with or without n-acetyl-l-cysteine (5 mM) for 24 h, were fixed with 1% paraformaldehyde, and immunohistochemical staining of acetylated histone 4 (H4) carried out. Acetylated H4 is shown by positive staining of nuclei on upper panel. The level of H4 positive staining was expressed as a percentage of total nuclei as demonstrated by Hoechst staining (not shown). The diagram demonstrates that PM_{10} upregulates the presence of acetylated histone in the nuclei as hypothesized. A clear role for oxidative stress in this effect of PM_{10} is demonstrated by the data showing that the effect of PM_{10} is prevented by the thiol antioxidant.

sponse of cells to endotoxin and oxidative stress [91]. Gilmour et al. [92] measured IL-8 mRNA expression and protein release in response to PM_{10} in human alveolar epithelial cells (A549) cells transfected with the E1A gene (E1A+ve). The activation of the AP-1 and NF-κB were also assessed. E1A+ve cells showed an enhanced IL-8 mRNA and protein response following treatment with H_2O_2 and PM_{10}, compared to E1A-ve cells. Enhanced induction of IL-8 in E1A+ve cells was accompanied by increases in AP-1 and NF-κB nuclear binding, and higher basal nuclear binding of these transcription factors. These data suggest that the presence of E1A primes the transcriptional machinery for oxidative stress signaling and therefore facilitates amplification of proinflammatory responses. Susceptibility to exacerbation of COPD in response to particulate air pollution may be conferred by this mechanism in COPD patients harboring E1A following adenoviral infection.

The AP-1 pathway

AP-1. AP-1 is an oxidative stress-sensitive transcription factor linked to inflammation, cell proliferation, and transformation, and is reviewed elsewhere [93].

AP-1 and particles. AP-1 is known to be stimulated in response to pathogenic particles such as asbestos [94], silica [72], and PM_{10} [92]. Recent work by Timblin et al. [95] demonstrated that air pollution particles and ultrafine carbon black at low concentrations upregulated mRNA levels of the early response protooncogenes c-jun, junB, *fos*-related antigen-1 (fra-1), and fra-2 in C10 cells, concurrent with cell proliferation. In contrast, high concentrations of ambient particulates caused transient increases in expression of fos and jun family members, along with expression of receptor-interacting protein, Fas-associated death domain, and caspase-8. In addition, ultrafine carbon black (UFCB) augmented mRNA levels of protooncogenes and apoptosis-associated genes, TNF receptor (TNFR)-associated death domain, and Fas. Interestingly, both nitric oxide (NO) and H_2O_2, reactive nitrogen and oxygen species generated during oxidative stress conditions, respectively, have been shown to increase c-jun and c-fos mRNA levels, nuclear proteins, and binding to AP-1 consensus sequence, accompanied by apoptosis in rat lung epithelial cells [96]. The accumulated evidence above suggests that air pollution particles may activate Fos and Jun protooncogenes in an oxidant-dependent manner, directing pulmonary epithelial cells to undergo either proliferation or apoptosis.

Histone acetylation

The role of histone acetylation. NF-κB binds to promoter sequences of inflammatory genes and initiates transcription to mRNA. However, the mechanism that facilitates the access of NF-κB and other transcription factors to the promoter sites, otherwise masked by the tight coiling of the DNA in the chromatin structure, is the process of histone acetylation and deacetylation [97]. The histone core of the chromatin structure, around which the nucleic acid helix is wound, is made up of four histone proteins (H2A, H2B, H3, and H4). When acetylated, these proteins relax the chromatin structure, thus exposing DNA to transcription factors [98]. Acetylation of histones is mediated by a number of transcription factors and coactivators that have intrinsic histone acetyltransferase (HAT) activity [99]. A number of histone deacetylase proteins initiate the deacetylation of histone, histone deacetylases (HDACs), creating a balance between HAT and HDAC that regulates activation and suppression of transcription [99].

NF-κB is known to associate with coactivators that have HAT activity such as CREB-binding protein (CBP) and p300, and their recruitment by transcription factors is an important link between promoter activation and transcriptional machinery [100]. Furthermore, NF-κB-associated co-activator proteins can remodel chromatin via inhibition of HDAC enzymes [101]. Thus NF-κB activation can alter both sides of the acetylation regulators to aid transcription.

Acetylation of histones has been associated with the transcription of a range of inflammatory mediators, including interleukin-8 (IL-8) [102], eotaxin, interleukin-1β (IL-1β), and granulocyte-macrophage-colony-stimulating factor (GM-CSF) [103], macrophage inflammatory protein 2 (MIP-2) [104], and interleukin-6 (IL-6) [100]. This acetylation can occur specifically at the promoter sites of these genes as shown by chromatin immunoprecipitation (CHiP) assays for IL-8 [105], cytochrome p450 1A1 (CYP1A1) [106], myeloperoxidase [107], and 15-lipoxygenase (15-lox-1) [108] gene promoters, indicating acetylation specificity.

HDACs not only cause the inhibition of gene transcription by deacetylating and therefore limiting coactivator access to target sites of DNA, but also directly affect the nuclear activity of transcription factors such as NF-κB. The duration of the NF-κB nuclear activation has been shown to be dependent upon the activity of HDAC3 [99], which provides an acetylation balance-dependent mechanism for the regulation of NF-κB mediated transcription.

Histone acetylation and PM_{10}. We have demonstrated that PM_{10}-stimulated increases in IL-8 protein release

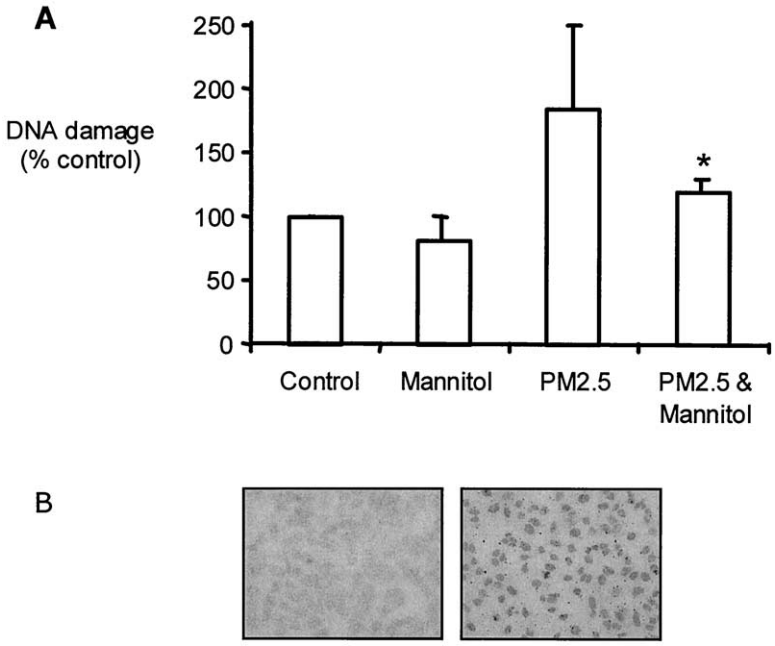

Fig. 4. Figure demonstrating oxidative DNA damage caused by PM_{10}. (A) DNA strand breakage by $PM_{2.5}$ in the presence or absence of the hydroxyl-radical scavenger mannitol. A549 human lung epithelial cells were treated for 3 h with $PM_{2.5}$ (125 μg/ml) suspended in Hank's Balanced Salt Solution, in the presence or absence of mannitol (25 mM). DNA damage was determined using alkaline single cell gel electrophoresis and quantified with software-assisted fluorescence microscopy analysis. *$p < .05$ vs. $PM_{2.5}$. Protection against the oxidative DNA damage is shown by the fact that mannitol protects. (B) Formation of 8-OHdG in A549 cells treated with $PM_{2.5}$. Cells were treated with PM (50 μg/ml) suspended in HBSS for 2 h and 8-OHdG was determined by immunohistochemistry using a monoclonal antibody to 8-hydroxy-2'-deoxyguanosine. Left panel = control; right panel $PM_{2.5}$.

from A549 cells is enhanced by co-treatment with the histone deacetylase inhibitor trichostatin (TSA) [109]. PM_{10} treatment also increased the activity of histone acetyltransferase, as well as the level of acetylated histone 4 in the nuclei of PM_{10}-exposed cells (Fig. 3C). The enhanced acetylation of Histone 4 was mediated by oxidative stress as shown by its inhibition with a thiol antioxidant. The acetylation of H4 mediated by PM_{10} was associated with the promoter region of the IL-8 gene. PM_{10}- and TSA- mediated increases in IL-8 and histone acetylation were associated with increases in NF-κB activation. These data suggest that the remodeling of chromatin by histone acetylation plays a role in the PM_{10}-mediated pro-inflammatory responses in the lungs. Acetylation occurs at the myeloperoxidase gene promoter by a mechanism reliant upon MAP kinase activation [107], a signal transduction pathway activated by diesel soot [110].

Since, as outlined above, the HDAC proteins play a role in the inactivation of gene transcription by deacetylation of histones and inactivation of NF-κB, impairment of HDAC activity would provide a mechanism for enhancement of NF-κB-mediated inflammatory gene transcription. The activity of HDAC 1 and 2 enzymes in lung phagocytes has been shown to be impaired as a result of cigarette smoke-induced oxidative stress [111], and histone acetylation appears to be a factor in asthma-related inflammation, as shown by the mechanism of action of effective asthma treatments [112]. PM_{10}-mediated oxidative stress also acts via this mechanism to enhance inflammation [109].

Oxidative stress and genotoxicity of PM: the role of hydroxyl-radicals

As discussed above, ROS have been identified as crucial mediators in the pathogenic effects of PM. With respect to their possible role in PM-induced lung cancer development [5], it is generally known that ROS are able to induce DNA damage, a process implicated in the initiation stage of carcinogenesis [113,114]. Various agents that are known to induce ROS formation have been shown to elicit DNA damage and have been associated with carcinogenicity, e.g., ionizing radiation, ultraviolet (UV) light, and asbestos [113,115]. Alternatively, genotoxic ROS may also be derived from inflammatory processes such as activation of the phagocytic oxidative burst. Preexistence of inflammatory lung diseases such as fibrosis and COPD has been associated with a higher risk of cancer development [116–118]. In vitro studies have demonstrated that neutrophils can induce DNA damage and gene mutations in epithelial lung

cells [119,120], and this is considered to be a major pathway for tumor formation in rat lungs in response to poorly soluble particles such as silica [118,121,122]. The most potent ROS to react with DNA is the hydroxyl radical ($^{\bullet}OH$), which generates damage to both DNA bases and deoxyribose residues [123]. Among these, 8-hydroxy-2'deoxyguanosine (8-OHdG) represents the best studied hydroxyl-radical specific DNA adduct [113]. Its formation is considered as a marker for cellular oxidative stress, and it has been demonstrated as a premutagenic lesion [113,124]. The association between oxidative stress and cancer is also indicated from observations that 8-OHdG levels are increased in cancerous tissues [125,126], although further studies are needed to prove causality. Recently established specific DNA repair knockout animals, such as ogg1 $-/-$ mice that specifically lack repair of 8-OHdG [127,128], represent promising models in this regard.

A role for $^{\bullet}OH$ generation in PM genotoxicity was already anticipated from incubation studies with naked DNA, although these methods were used to determine radical activity of PM. For instance, DNA strand breakage by PM, as determined by a plasmid-unwinding assay, can be inhibited with the $^{\bullet}OH$ scavenger mannitol [129,130]. Furthermore, the induction of 8-OHdG is observed upon incubation of PM or PM-model compounds such as coal and oil fly ashes with 2'-deoxoguanosine or with calf thymus DNA [131,132]. Formation of $^{\bullet}OH$ by PM can also be shown directly in electron spin resonance (ESR) studies using the spin trap 5,5-dimethyl-1-pyrroline-N-oxide [130,132,133]. With this method it can be demonstrated that various size fractions of particulate matter, including total suspended PM (TSP), PM_{10}, $PM_{10\text{-}2.5}$ (coarse PM), $PM_{2.5}$, and PM_1 are all capable of generating $^{\bullet}OH$ in suspension [134]. Moreover, ESR measurements of dry PM samples also show spectra indicative of semiquinone radicals similar to those found in cigarette tar samples, suggesting that H_2O_2 formation via redox cycling of these compounds can contribute to $^{\bullet}OH$ generation [135]. Induction of both DNA strand breakage and 8-OHdG by PM has also been shown in lung epithelial cells [130–132,135]. Inhibition of PM-induced DNA strand breakage, as for instance observed with mannitol (Fig. 4A), tetramethylthiourea (TMTU), dimethyl sulfoxide (DMSO), catalase, or the iron chelator deferoxamine thus also indicates a role for Fenton-derived $^{\bullet}OH$ in these cellular systems [132,135]. Further support for this mechanism comes from observations that PM (Fig. 4B) as well as coal and oil fly ashes, induce 8-OHdG in lung epithelial cells, which in turn can be inhibited by deferoxamine [130–133]. Importantly, 8-OHdG can be considered as a general marker of cellular oxidative stress [124], so its induction could also be seen as evidence of intracellular formation of ROS in relation to the proposed mechanisms of inflammation by PM [23,26,89,136]. Notably, the acellular assays (i.e., naked DNA, ESR) show $^{\bullet}OH$ formation by PM in the absence of H_2O_2, but a clear enhancement of this effect was seen upon addition of physiologically relevant (i.e., micro-molar) levels of H_2O_2 [132]. The following observations:

1. PM elicit H_2O_2 release by neutrophils [132].
2. Activated neutrophils induce 8-OHdG in epithelial cells in vitro [120].
3. Increased levels of lung H_2O_2 seen in inflammatory lung diseases such as COPD [137,138] suggest that the intrinsic capacity of inhaled PM to induce cellular oxidative stress and associated DNA damage may be enhanced in such compromised individuals, which deserves further investigation.

CONCLUSION

The foregoing review has highlighted the important role that oxidative stress plays in the adverse effects of PM_{10}. The central hypothesis is that inflammation drives the effects of PM_{10} on exacerbations of lung disease, and that pulmonary inflammation may also underlie the cardiovascular effects [15] although the mechanisms of the cardiovascular effects of PM_{10} are not well understood. There are several components commonly found in PM_{10} that are likely to be involved in the induction of oxidative stress, including transition metals, ultrafine particle surfaces, and organic compounds. Oxidative stress arising from PM_{10} activates a number of redox-responsive signaling pathways in lung target cells that are involved in expression of genes that play a role in responses relevant to disease and pathological change, including MAPKs, NF-κB, AP-1, and histone acetylation (summarized in Fig. 1). Oxidative stress from particles is also likely to play an important role in the carcinogenic effects associated with PM_{10}, and hydroxyl radicals from PM_{10} cause DNA damage in vitro.

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