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World Trade Center (WTC) dust exposure in mice is associated with inflammation, oxidative stress and epigenetic changes in the lung

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ABSTRACT

Exposure to World Trade Center (WTC) dust has been linked to respiratory disease in humans. In the present studies we developed a rodent model of WTC dust exposure to analyze lung oxidative stress and inflammation, with the goal of elucidating potential epigenetic mechanisms underlying these responses. Exposure of mice to WTC dust (20 μg, i.t.) was associated with upregulation of heme oxygenase-1 and cyclooxygenase-2 within 3 days, a response which persisted for at least 21 days. Whereas matrix metalloproteinase was upregulated 7 days post-WTC dust exposure, IL-6RA1 was increased at 21 days; conversely, expression of mannose receptor, a scavenger receptor important in particle clearance, decreased. After WTC dust exposure, increases in methylation of histone H3 lysine K4 at 3 days, lysine K27 at 7 days and lysine K36, were observed in the lung, along with hypermethylation of Line-1 element at 21 days. Alterations in pulmonary mechanics were also observed following WTC dust exposure. Thus, 3 days post-exposure, lung resistance and tissue damping were decreased. In contrast at 21 days, lung resistance, central airway resistance, tissue damping and tissue elastance were increased. These data demonstrate that WTC dust-induced inflammation and oxidative stress are associated with epigenetic modifications in the lung and altered pulmonary mechanics. These changes may contribute to the development of WTC dust pathologies.

1. Introduction

The collapse of the World Trade Center (WTC) in New York City on September 11, 2001 resulted in high concentrations of a complex mixture of airborne pollutants, consisting of coarse and fine particles from building materials and combustion products in the ensuing plume. This included, but was not limited to cement, glass fibers, asbestos, metals, organic and inorganic particles and gases. Analysis of WTC dust revealed the presence of particles small enough to penetrate deep into the lungs of first responders, recovery workers and residents, reaching the distal airways and alveoli (Lioy et al., 2002; Gavett, 2003; McGee et al., 2003; Lioy and Georgopoulos, 2006). Evidence suggests a link between exposure to WTC dust-derived airborne materials and respiratory disease (Guidotti et al., 2011; Wisnivesky et al., 2011; Berger et al., 2013). Upper and lower respiratory tract pathologies including chronic rhinosinusitis, bronchitis, asthma and bronchiolitis have been reported in individuals exposed to WTC dust (Guidotti et al., 2011). Sarcoid-like granulomatous inflammation has also been noted in rescue and recovery workers (Izbicki et al., 2007; Crowley et al., 2011). To characterize these responses, we developed an animal model of WTC dust exposure, with the goal of elucidating potential epigenetic mechanisms underlying disease pathogenesis.

Epigenetic changes encompass an array of molecular modifications to both DNA and chromatin. The most extensively investigated modifications are DNA methylation at the carbon-5 position of cytosine in CpG dinucleotides, and alterations in chromatin packaging of DNA by post-translational histone modification, such as methylation and acetylation (Jirtle and Skinner, 2007). These are known to be involved in regulating macrophage activation and inflammatory gene expression (Jirtle and Skinner, 2007; Kapellos and Iqbal, 2016). Exposure to diesel exhaust particles, cigarette smoke and ozone has been reported to cause epigenetic changes in the respiratory tract, which have been implicated in the development of inflammatory lung diseases (Rajendrasozhan et al., 2008; Baccarelli et al., 2009; Ho et al., 2012). DNA methylation and histone modifications are thought to be the major epigenetic mechanisms leading to transcriptional silencing of genes relevant to the development of airway inflammatory and allergic diseases following

Abbreviations: WTC, World Trade Center; i.t., intratracheal; i.p., intraperitoneal; HO-1, heme oxygenase-1; COX-2, cyclooxygenase-2; MMP-12, matrix metalloproteinase-12; BAL, bronchoalveolar lavage; PEEP, positive end expiratory pressure; COPD, chronic obstructive pulmonary disease; K, lysine.

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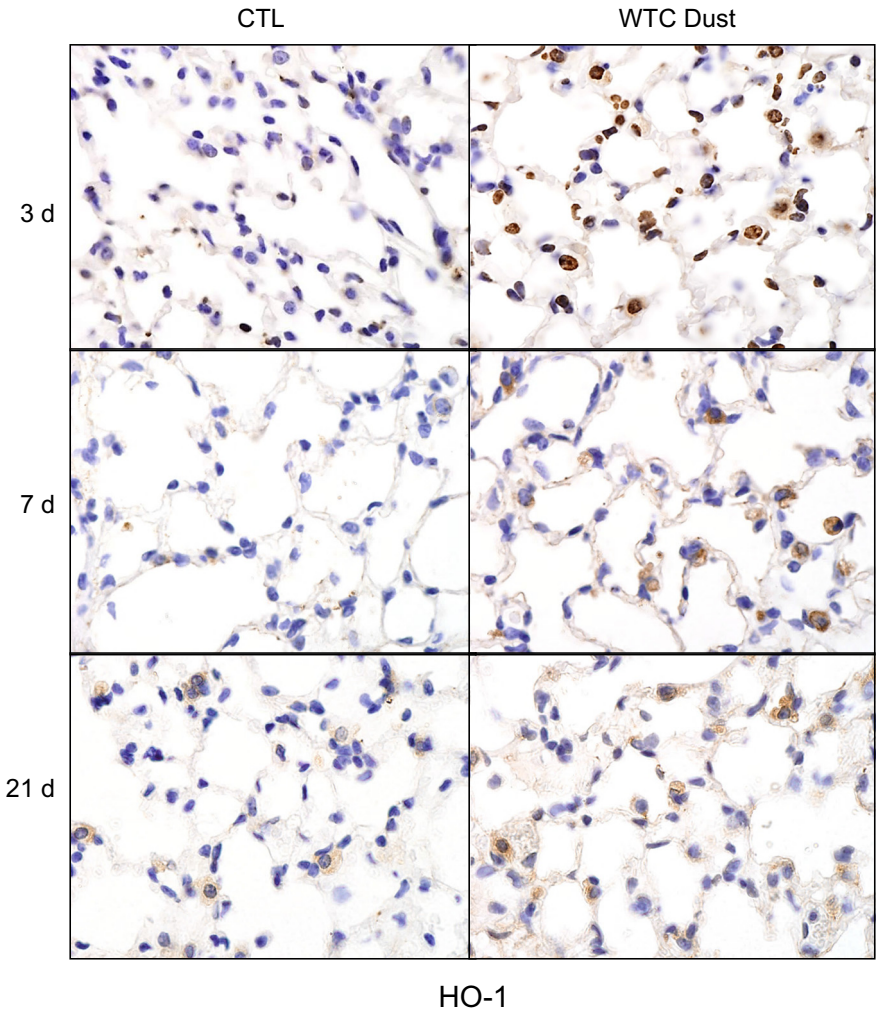

Fig. 1. Effects of WTC dust on HO-1 expression. Tissue sections, prepared 3 d, 7 d and 21 d after exposure of mice to PBS (CTL) or WTC dust, were stained with antibody to HO-1. Binding was visualized using a peroxidase DAB substrate kit. One representative section from 4–6 mice per treatment group is shown (Original magnification, ×600).

environmental exposures (Adcock et al., 2007; Kuriakose and Miller, 2010; Ho et al., 2012; Lepeule et al., 2012; Tzouvelekis and Kaminski, 2015).

In the present studies, we analyzed the effects of WTC dust exposure on pulmonary inflammation and oxidative stress, and on lung function in an experimental rodent model. We also determined if WTC dust-induced lung changes were associated with DNA methylation and histone modifications. Elucidating mechanisms underlying WTC dust disease may be useful in understanding inflammatory disease pathogenesis in humans and developing efficacious therapeutic approaches.

2. Materials and methods

2.1. Materials

A bulk sample of settled WTC dust was collected from a single location on Market Street in New York City in a protected area near ground zero on day 5 after the attack. The physical characteristics and chemical composition of the sample have been described previously (Lioy et al., 2002). The sample represented total settled dust and was used in these experiments without pre-sizing or other modifications.

2.1.1. Animals, treatments and sample collection

Female specific pathogen-free C57BL6/J mice (8–10 weeks, 17–20 g) were obtained from The Jackson Laboratories (Bar Harbor, ME). Animals were housed in filter-top microisolation cages and maintained on food and water *ad libitum*. All animals received humane care in compliance with the institution's guidelines, as outlined in the *Guide for the Care and Use of Laboratory Animals*, published by the National Institutes of Health. Animals were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) and then placed on a tilting rodent work stand (Hallowell EMC, Pittsfield, MA) in a supine position

Table 1
Quantification of immunohistochemistry.

	Time after treatment	CTL	WTC dust
HO-1	3 d	0.38 ± 0.13	1.00 ± 0.16[a]
	7 d	0.25 ± 0.14	0.42 ± 0.08[b]
	21 d	0.25 ± 0.14	0.10 ± 0.10[b]
MR-1	3 d	3.13 ± 0.13	1.50 ± 0.42[a]
	7 d	1.88 ± 0.66	0.75 ± 0.17
	21 d	1.30 ± 0.44	2.20 ± 0.52[c]
COX2	3 d	2.25 ± 0.48	2.40 ± 0.25
	7 d	1.63 ± 0.32	2.08 ± 0.44
	21 d	1.75 ± 0.25	2.60 ± 0.60

Alveolar macrophages (AM) staining positive for heme oxygenase (HO)-1 and mannose receptor (MR)-1, and Type II cells staining for cyclooxygenase (COX)-2, were enumerated in 450 fields (40)/lung section and assigned a staining intensity score of 0 to 3 (0, no staining; 0.5, minor staining; 1, light staining; 2, medium staining; 3, dark staining). Values are the mean intensity score ± SE of 4–6 mice/treatment group. Data were analyzed by 2 way ANOVA.

[a] Significantly different ($p < 0.05$) from CTL.
[b] Significantly different ($p < 0.05$) from 3 d.
[c] Significantly different ($p < 0.05$) from 21 d.