Article

Indoor PM10 Upregulates Pro-inflammatory Mediators in Bronchial Epithelial Cells

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Abstract: Indoor dusts are collectively formed from anthropogenic and atmospheric activities. Particle matter 10 (PM10) is inhalable and causes significant inflammation by interaction with the pulmonary epithelial barrier. The mediators involved in bronchial epithelial cells response to dust are remined unknown. The air-liquid interface of our lung on chip model was exposed to indoor dust collected from highly polluted houses in Delhi, India. The media were collected after 4 days and cytokine levels were measured. We found that the concentration of IFNα, IFNγ, Interleukin-6 (IL-6), IL1b, TNFa, and Granulocyte monocyte colony stimulating factor (GM-CSF) were significantly increased after exposure to indoor dust. IFN type I pathways were a major response from dust exposure. Further investigation is needed to determine the mechanism of action and targets of dust in bronchial epithelial cells.

1. Introduction

Indoor exposure to aerosolized particulate matter up to ten micrometers in size (PM10) is a significant respiratory health concern, especially in urban environments and in low-middle income countries with poor air quality [1]. Indoor PM10 may come from indoor activities like cooking, or it may be transported from outdoor air pollution [1, 2]. In fact, PM10 is spatially and temporally correlated to outdoor anthropogenic activity [2]. During inspiration, PM10 is brought into the respiratory tract through airflow convection. Given its size, ≤ 10μm in aerodynamic diameter, PM10 deposits in bronchial airways through sedimentation or inertial impaction leading to the subsequent development of pulmonary disease [3]. The link between increased air pollution and the development of pulmonary pathology is attributed to direct oxidant release from organic and metal components as well as indirect oxidative cellular responses. Production of reactive oxygen species and oxidative stress initiates an inflammatory cascade leading to cellular apoptosis [4]. PM10 penetrates the mucosal layer of bronchial airways leading to localized epithelial damage which is subsequently amplified by cytokine production [5].

Interferon-α is a type I interferon (IFN 1) implicated in regulation of inflammation in viral infections and immune disorders [6]. Recently, we investigated the role of Mycobacterial cell wall microparticles on normal human bronchial epithelial (NHBE) cells and found a differential

overexpression of IFN 1 pathway proteins and genes in the exposed NHBE cells as compared to unexposed cells suggesting an innate IFN I mediated immune response in NHBE [7]. We hypothesized that IFN I could also have a central role in mediation of innate immune response in NHBE cells following exposure to indoor PM_{10}. Literature review revealed no previous study on the role of the IFN I pathway in bronchial epithelial responses to respirable PM_{10} exposure. In this study, we explored the role of IFN 1 in NHBE cells following exposure to PM_{10} dust in a highly polluted urban setting.

2. Materials and Methods

Indoor PM_{10} was collected from a residential setting (patient houses) in Delhi, India on 37mm PTFE membrane filters using a vacuum pump for 72 hours. The samples were analyzed using Kevex™ Energy Dispersive X-Ray Spectrometer to identify elemental composition of PM_{10} at Environmental Protection Agency (EPA)'s National Exposure Research Laboratory, Research Triangle Park, NC.

Extracted PM_{10} dust was then exposed to NHBE in the lung-on-chip model (LOCM). LOCM is a biological lung model designed to simulate a physiological human airway with two cell line layers; primary NHBE isolated from lungs rejected for transplant at University of Miami at the top of the membrane and endothelial cells (Human Lung Microvascular Endothelial Cells, Lonza, Walkersville, MD) at the bottom. A transwell® polyester membrane cell culture insert (12 mm diameter, 0.4 µm pore size; Corning Life Sciences, Amsterdam, The Netherlands) was used for cell culturing as discussed elsewhere [7, 8].

An air channel was connected to the epithelial layer while a media channel was connected to the endothelial layer as shown in Figure 1. PM_{10} exposure was set to a concentration of 500 ppm in a 55.7µL solution to 944.3 µL of media and was added to ALI air-liquid interface. After 4 days of exposure, the medium was collected and cytokines (IL-1RA, IL-2, , IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8,IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, GM-CSF, Eotaxin, IFN-α, IP-10, IFN-γ, MCP-1, IL-1β, MIG, MIP-1α, , MIP-1β, RANTES, TNF-α) were measured using Human Magnetic 25-Plex Kit (Thermofisher, Catalog number: LHC0009M). Data were reported as mean ± standard error (SE), and each experiment was repeated 3 times. A paired *t*-test was used to compare two means. A $p < 0.05$ was defined as statistically significant. The String dataset was used to determine the protein-protein interaction (PPI) networks [9]. The PPI was limited to "Homo sapiens" and interaction score more than 0.4 were used to develop the network.

3. Results

The indoor dust sample had a concentration of 315078 ng/m3 with 64.97% of the PM_{10} containing various elemental species. The ten most abundant elements found in the sample were silicon (34.72%), iron (12.50%), aluminum (12.49%), calcium (12.11%), potassium (8.79%), sulfur (5.67%), lead (4.89%), zinc (3.96%), chloride (2.36%), and magnesium (2.54%). In the LOCM experiment, the collected media showed a statistically significant increase in IFNα, IFNγ, Interleukin-6 (IL-6), IL1β, TNFα, and Granulocyte monocyte colony stimulating factor (GM-CSF) in LOCM exposed to PM_{10} as compared to control as shown in Figure 1. The rest of cytokines were not significantly changed between two groups. In the PPI network, IFNα functionality is associated with TNFα, IL6, IFNγ, and GM-CSF as depicted in Figure 1.

3.1. Figures
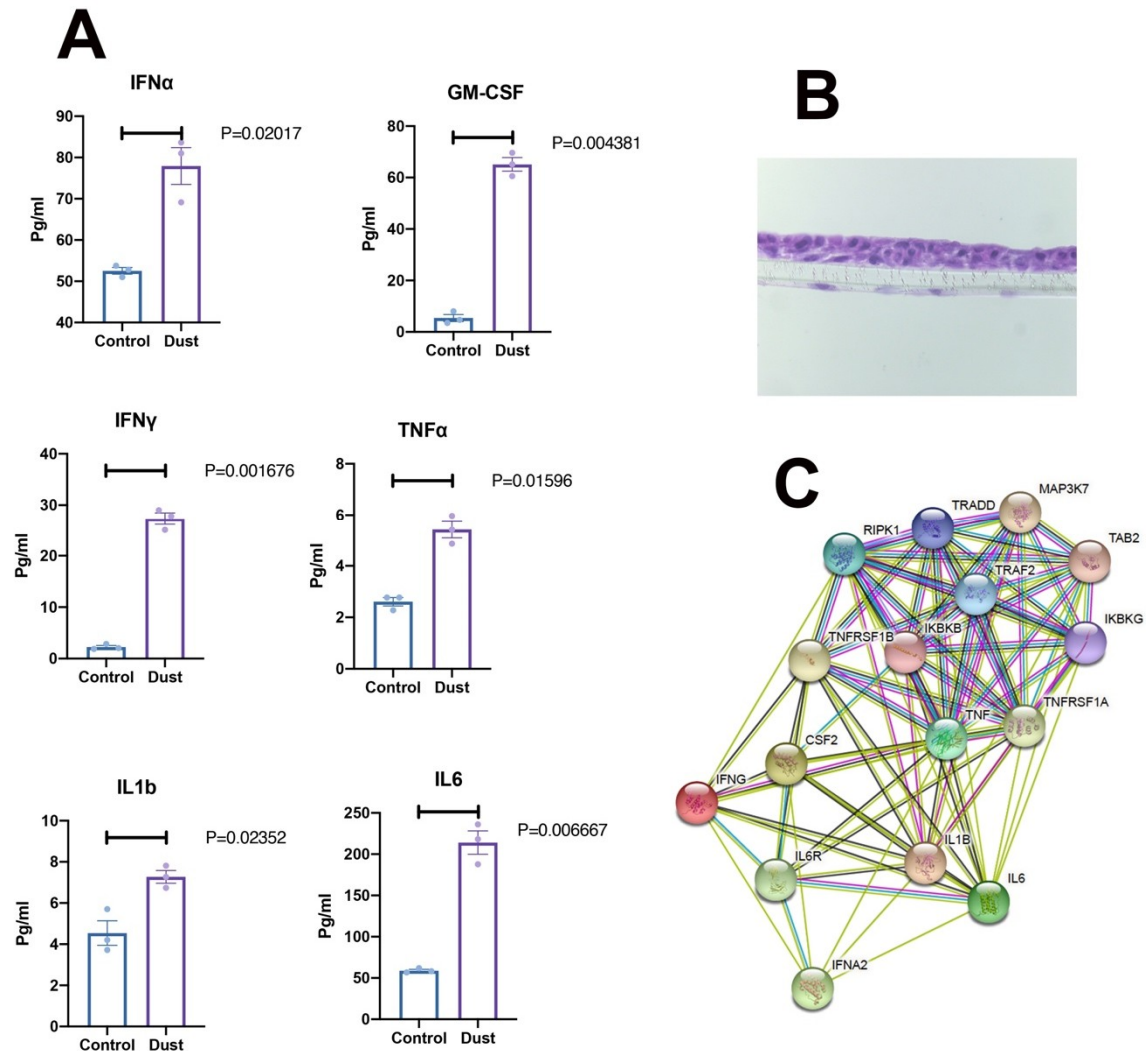
Figure 1. (a) Shows cytokines with significant expressions in media after exposure of ALI with indoor PM_{10}. Dust (PM_{10}) was exposed to the ALI of lung model as shown in **(b).** Top layer is mature pseudostratified bronchial epithelial cells, membrane in the middle and pulmonary small vessels endothelial in the bottom of the membrane. **(c)** Shows String interaction network of cytokines found with significant increase in PM_{10} exposure and their first level of protein interaction in the network.

4. Discussion

This study found a statistically significant increase in multiple cytokines in a lung-on-chip model after exposure to indoor particles composed of various chemical elements (most commonly silicon). Specifically, INF-α concentrations increased relative to non-exposed lung models (p <0.02) along with an associated increase in other cytokines (IFNγ, IL-6, TNF-α , and GM-CSF) suggesting a complex immune response involving the interplay between multiple related cytokines.

IFNα is produced by almost all body cells including epithelial cells. IFNα is known as a moving target due its ability to mediate opposing effects [10]. While IFNα demonstrates an anti-inflammatory role in virally infected epithelium and multiple sclerosis, it is also the potential orchestrator of pathological inflammation in certain immune disorders such as systemic lupus erythematosus [6]. Its dichotomous role in inflammation is explained by its opposing effects on helper T cell subtypes, TH1 and TH2. Previous studies show IFNα can enhance TH1 response via activation of inflammatory cytokines including IL12, IL18 and caspase1 and inhibit TH2 via activation of   IL4 [11, 12].

TH1 mediated response is further reinforced by TH1 secreted IFNγ which increases naïve T-cell responsiveness to IL12 [12]. Our study revealed elevated levels of IFNγ (p< 0.001), suggesting a complementary role of IFNγ in propagation of TH1 response.

There is evidence suggesting that IFNα mediates an increases in IL6, a cytokine which was elevated in our study (p<0.01), in the setting of toll-like receptor (TLR) 8 activation in neutrophils [13]. TLR8 is an endosomal receptor known to recognize single-stranded DNA viral proteins[13]. Given that TLR8 are also present in bronchial epithelial cells suggests a possible mechanism of IL6 activation via IFNα.   Furthermore,   the process of IL6 activation is potentiated by the TH1 committed pro-inflammatory cytokine tumor necrotizing factor (TNF)α, which was also elevated in our study (p<0.01) [13].

Our proposed mechanism for the sequence of cytokine release is listed as the following. Indoor aerosolized particle matter first deposits in bronchial airways. IFNα is produced by bronchial epithelial cells which leads to TH1 activation and TH2 inhibition. Activation of TH1 cells leads to the subsequent release of cytokines IFNγ and TNFα. IFNγ further increases naive TH1 cell activation and mediates increases in IL6 via action of TLR 8 found in bronchial epithelial cells. Similarly, TNFα potentiates IL6 signaling.

The mechanism of PM10 induced inflammation is largely still unknown, but the inflammatory host response to aerosolized particulate matter has been previously studied. Bossmann *et al.* studied the inflammatory response in peripheral blood mononuclear cells after exposure to PM10 particles and found notable increases in TNF α, IL-6, and IL-1β[14]. Park *et al.* studied human dermal fibroblast response to PM10 and observed increases in IL-1β, IL-6, IL-8 and IL33 [15]. Becker *et al.* found that alveolar macrophage response to virally infected lung epithelial cells was altered in response to PM10 exposure [16]. *Ramage et al.* found increased expression of C-reactive protein and heat-shock protein-70 secondary to oxidative inflammatory response in lung epithelial cells after treatment with PM10 [17].

Recently, we found overexpression of IFN1 proteins in NHBE cells in response to mycobacteria cell wall particles, which prompted the development of this study to look for a similar response in the setting of PM10 exposure [7]. This study was the first to demonstrate a significant pro-inflammatory IFNα mediated inflammatory cascade in response to PM10. However, further studies are needed to identify the presence of TH1 cell lines, mRNA expression of cytokines, and

gene/protein expression of cytokines to fully elucidate our proposed mechanism. Content of the PM_{10} was analyzed for the percent component of each chemical element, but individual chemical elements were not assessed for biological response. Additional studies are needed to assess the biological response of each component to increase clinical applicability.

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Conflicts of Interest: All authors disclose no conflict of interest.

Appendix B

All appendix sections must be cited in the main text. In the appendixes, Figures, Tables, etc. should be labeled starting with 'A', e.g., Figure A1, Figure A2, etc.

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