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Particulate Matter Containing Environmentally Persistent Free Radicals and Adverse Infant Respiratory Health Effects: A Review

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Abstract

The health impacts of airborne particulate matter (PM) are of global concern, and the direct implications to the development/exacerbation of lung disease are immediately obvious. Most studies to date have sought to understand mechanisms associated with PM exposure in adults/adult animal models; however, infants are also at significant risk for exposure. Infants are affected differently than adults due to drastic immaturities, both physiologically and immunologically, and it is becoming apparent that they represent a critically understudied population. Highlighting our work funded by the ONES award, in this review we argue the understated importance of utilizing infant models to truly understand the etiology of PM-induced predisposition to severe, persistent lung disease. We also touch upon various mechanisms of PM-mediated respiratory damage, with a focus on the emerging importance of environmentally persistent free radicals (EPFRs) ubiquitously present in combustion-derived PM. In conclusion, we briefly comment on strengths/challenges facing current PM research, while giving perspective on how we may address these challenges in the future.

Keywords

Particulate Matter; Environmentally Persistent Free Radicals; Infant; Respiratory Health; Immunomodulation

PARTICULATE POLLUTION

Airborne particulate matter (PM) is generated by a variety of sources including vehicular exhaust, flaring of hydrocarbons at refineries, coal burning at power plants, and thermal treatments of hazardous wastes. PM is often categorized according to mean aerodynamic diameter with sizes ranging from coarse particles (2.5–10 μm in diameter) to the smaller fine (<2.5 μm) and ultrafine (<0.1 μm) particles. The smaller classes of PM are more apt to cause respiratory toxicity and dysfunction due to their propensity to deposit deep in the lower airways and alveoli [1]. Owing to the increasing knowledge of the detrimental health consequences of exposure to PM, the United States Environmental Protection Agency (US EPA) through the Clean Air Act has set National Ambient Air Quality Standards (NAAQS). The current recommendations for PM_{2.5} and PM₁₀ are 35 and 150 $\mu\text{g}/\text{m}^3$ within a 24-h

period, respectively, whereas the annual limit of exposure for PM_{2.5} is 15 µg/m³ [2]. The World Health Organization (WHO) is more stringent with daily exposure limits for PM_{2.5} and PM₁₀ with concentrations set at less than 25 and 50 µg/m³, respectively; and an annual PM_{2.5} limit at less than 10 µg/m³ [3, 4]. There are no current recommendations for daily or annual exposure limits for ultrafine particles either nationally or globally. Astoundingly, the WHO estimates that if air quality guidelines were met, an estimated 1.09 million premature deaths would have been prevented in 2008 [5,6].

Although extensive research has focused on outdoor air pollution, indoor air pollution (IAP) also poses a significant health risk. Most IAP is a mixture of cigarette smoke and/or biomass combustion from cooking stoves—the products of which contain similar organic species as found in outdoor air pollution. Those with the greatest risk from exposure to IAP are, as expected, those who are exposed for the longest period of time (i.e., women, infants, young children, and the elderly) and/or those with underlying immune/pulmonary/cardiovascular disorders. Worldwide, exposure to IAP contributes to nearly two million premature deaths annually due to acute lower respiratory tract infections in children under five, chronic obstructive pulmonary disease in adults (equivalent to a lifetime of cigarette smoking), and respiratory tract cancers in adults [7, 8]. There are additional known and suspected health risks associated with exposure to IAP such as low birth weight, anemia, stunted growth in infants and children [9, 10], interstitial lung disease, increased incidence of tuberculosis [11], cardiovascular disease [12, 13], and cataracts [14,15]. IAP is thought to have such an impact on public health that the U.S. government has committed more than \$50 million to alleviate its negative consequences through the Global Partnerships Initiative and contributions to NIH's ongoing research funds [7]. This article briefly reviews the health effects associated with combustion generated PM in infants focusing on the respiratory effects and proposes a new mechanism for their role in respiratory disease.

ENVIRONMENTALLY PERSISTENT FREE RADICALS: RESPONSIBLE FOR THE HEALTH IMPACTS OF PM?

It has been demonstrated that size, shape, and composition of PM are important factors in determining toxicity [16, 17]. In addition, organic pollutants are often associated with PM [18]. It was originally presumed that organic pollutants must be weakly associated (i.e., Van der Waals, hydrogen bonds) with PM; however, studies have demonstrated that organic pollutants can be surface-bound to PM and stubbornly resistant to removal, implying a much stronger chemical bond [19]. Indeed, several pioneering studies by our colleagues demonstrated that particular aromatic hydrocarbons chemisorb to the surface of PM through transition metal oxides [20–23]. This environmental phenomenon results in the production of a surface-stabilized free radical species. These radical species are very weakly reactive with O₂ giving the ability to persist in ambient air for extended periods of time (hours-months) and at least 12 h in biological fluids (Figure 1). The stability of the radical is partly due to stabilization on the surface of the particle, whereas the persistency in biological systems is at least partially a result of a cyclic mechanism involving the biological regeneration of reactive oxygen species (ROS) [24]. These environmentally persistent free radicals (EPFRs) chemisorbed to PM are mostly semiquinone-type radicals similar to those found in cigarette smoke [25], but we have also identified them associated with PM from almost all combustion sources. Furthermore, there have been a number of studies concerning radicals associated with environmental PM [26–29]. Large quantities of similar semiquinone radicals have been found in PM collected from cities [22], with average radical concentrations being ~10× higher for industrialized, highly populated locations than less densely populated areas (unpublished observations). Radical concentrations in these environmental samples, measured in spins/gram, were typically 2–10× higher than in cigarette tar [30, 31], and each sample exhibited an electron paramagnetic resonance (EPR)

spectrum with a g value of ~ 2.0033 – 2.004 , which is typical of oxygen-centered or oxygen-containing EPFRs. Owing to the recent discoveries of EPFRs, understanding of their impact on health is in its infancy. Their radical and regenerative nature, however, suggests the potential for causing dire health consequences, highlighting why further investigation is of grave importance. We have been working to understand the mechanism of toxicity and biological activity of EPFR-containing particles using in vitro and in vivo approaches; and thus, it is the focus of this review even though we appreciate the chemical complexity of PM and its many components (e.g., organics, particulates) that have also been shown to contribute to damaging health effects.

INFANTS: A SPECIAL POPULATION AT RISK

PM and Feto-Infant Growth and Morbidity

Epidemiological data support a link between elevations in PM and exacerbation of respiratory disease in adults [32, 33], and it is now widely accepted that exposure to PM contributes to inflammatory airways diseases, such as asthma [34]. More recently, investigators have begun to examine the effects of air pollution on infant/child health. Although the majority of these studies are epidemiological and typically related to tobacco smoke, they demonstrate a significant association between exposure to PM and feto-infant morbidity outcomes such as low birth weight and preterm birth [35, 36]. One inhalation study done in healthy humans concluded that particle deposition rate, when normalized to surface area of the lung, was 35% higher in children compared to both adolescents and adults [37]. Additional concerns, however, include increased incidence of placental abruption in women exposed to PM levels that were well below EPA limits ($PM_{2.5} > 10.97 \mu\text{g}/\text{m}^3$ and $PM_{10} > 25.04 \mu\text{g}/\text{m}^3$) compared to those *not* exposed to those levels of PM [35]. Racial/ethnic disparities in the effects of PM on feto-infant morbidity outcomes were also observed with African American infants being most at risk, suggesting that other factors such as social economic status, education, or even surrounding environment may also be an important confounding factor. A study in Korea suggested that area level social economic status modified the effects of PM on preterm births [38], where those who resided in low income areas had a higher probability of having a preterm birth when PM levels increased by $10 \mu\text{g}/\text{m}^3$, specifically if these elevated exposures occurred in the second trimester. More alarmingly, risk of respiratory death more than doubles for infants between the age of 7–12 months if exposure to elevated levels of PM occurred during the previous 6 months prior to death [39].

PM and Asthma

Since PM affects the growth and development of the embryo/fetus, it is not surprising that infants represent a particularly susceptible population to the effects of PM as they take in more air per body weight, their lungs are still developing (i.e., at birth only 6.7–17.5% of the alveoli are formed [40]), and they have immature immune systems. One of the main risk factors for adult asthma is early life insults to the lung (both structurally and immunologically) brought upon by environmental exposures [41]. Because of this, there has been a surge in the number of studies attempting to understand the role of environmental factors (i.e., particulate pollution or respiratory tract viral infections) in determining risk to develop asthma/wheeze and other child respiratory health concerns (Figure 2A). Unfortunately, still very few studies explore the interplay between all three factors (Figure 2B).

A large amount of epidemiological data exist linking elevated levels of PM and rate of hospitalization for children with adverse pulmonary events such as asthma exacerbations [42–46]. Indeed, several studies have demonstrated a positive correlation between PM

exposure and lung function changes in children including decreases in forced vital capacity (FVC), forced expiratory flow (FEF), and forced expiratory volume (FEV₁) [47–50]. Furthermore, it has been shown that exposure to fine and ultrafine PM affects lung development, resulting in functional deficits in the adult [51,52]. To further support the link between early life exposure to PM and its effect on subsequent adult respiratory health, histopathological lung changes such as septal enlargement, fibrosis, glandular hyperplasia, and goblet cell metaplasia have been observed in autopsies of children *less than 5 years of age* that had been exposed to biomass fuel [53]. In the majority of these children, these data were consistent with pulmonary changes reminiscent of bronchitis.

PM and Viral Infection

A few epidemiological studies have associated risk for and severity of respiratory tract viral infection with living near a source of traffic pollution, observing that infants who lived closer to highways had an elevated risk of being hospitalized for severe influenza or respiratory syncytial virus (RSV)-induced bronchiolitis [54, 55]. Moreover, elevated levels of PM have been attributed to increased risk of postnatal mortality from respiratory tract viral infections [56, 57]. Currently, the RESPIRE study is the only randomized, controlled trial that has documented the benefits of reducing IAP on child pneumonia [58]. Data from this study suggest that *up to 90%* of exposure reductions are *required* to attain significant decreases in pneumonia risk and even a 22% risk reduction requires a 50% decrease in exposure [59], highlighting the dramatic impact of PM on child health. Similar data are obtained when looking specifically at exposure to diesel exhaust particles (i.e., increased susceptibility, viral burden, and disease severity to respiratory viral infections including influenza and RSV) [60–63].

Not only is there a clear association between increased ambient PM and hospitalization of childhood bronchiolitis and respiratory disease, but it has also been demonstrated that there is a corresponding increase in the amount of healthcare dollars spent annually [64]. Increases in healthcare cost are attributable to the increase in the amount of cases and the enhanced severity of bronchiolitis due to increased PM exposure. In fact, it is estimated that reducing levels of PM to just 7% below the current annual standard would save the U.S. approximately \$15 million annually in healthcare costs [64], giving us not only a *medical* but an *economic* impetus to reduce combustion-derived PM emissions while also encouraging further investigations on how PM enhances infectious disease severity.

Limited mechanistic research that does exist for infants/children demonstrate that exposure to combustion-derived PM increases susceptibility to respiratory tract viral infections by dampening the initial immunological response while skewing the pulmonary immune response toward a T helper type 2 cell (Th2) response [65]—a response often seen in allergy with corresponding antibody production that is predominantly immunoglobulin type E (IgE), followed by an augmentation of downstream morbidities [66–69]. Altogether, these data suggest that EPFR-containing PM (i.e., roadway PM and diesel exhaust particles) (1) may be responsible for the increased risk for respiratory viral susceptibility and enhanced disease and (2) has a significant impact on the developing respiratory tract. Hence, the role of exposure to this class of PM on infantile susceptibility to viral infection is an area that requires much more attention. Much of the current data are derived primarily through epidemiological studies in which associations between PM and enhanced susceptibility to viral infections are suggested. Unfortunately, studies that further delve into the underlying mechanism of this phenomenon in an *age appropriate animal model* are severely limited.

Use of an Age-Relevant Model Is Critical

Collectively, the aforementioned observations suggest that there are sensitive life stages during which exposure to elevated PM has particularly devastating and long-term effects. Furthermore, the different toxicokinetic and toxicodynamic properties due to age makes studying an age-relevant model to understand the mechanistic basis for enhanced respiratory morbidity and mortality crucial.

In age-relevant rodent models, we have observed specific adverse pulmonary effects including airway hyperresponsiveness, airway remodeling, lung inflammation characterized by influx of neutrophils in bronchoalveolar lavage fluid (BALF), peribronchial associated lymphoid tissue, and increased smooth muscle mass following acute (7-day) EPFR inhalation exposure [70]. These characteristics correspond to noneosinophilic, severe asthma in humans. Even more strikingly, we observe lung function deficits that persist into early adulthood (Figure 3) [70], which recapitulates what is seen in humans [71]. In comparison, adult rodents acutely exposed to combustion-generated ultrafine particles do not exhibit persistent changes in airway function, reinforcing the need to study these effects in an age appropriate model.

Clearly, it is important to consider the developmental status of the lung in models used to study the effects of PM and how interruption/deviation from normal maturation could be responsible for much of the long-term effects observed in humans. Although relatively little is known about the mechanism involved in PM-induced airways dysfunction, our data suggest that airway remodeling plays a major role in long-term dysfunction [70,72].

MECHANISMS INVOLVED

Oxidative Stress

There are many observed and hypothesized mechanisms to explain PM-induced respiratory effects. One of the most popular involves PM-driven oxidative stress [73–78]. Oxidative stress results from an impaired ability of antioxidants to protect from harmful ROS. Some of these include superoxide ($O_2^{\bullet-}$), hydroxyl radical ($HO^{\bullet}$), and hydrogen peroxide (H_2O_2). As discussed above, during combustion processes many different types of molecular pollutants are able to bind to transition metal-containing particles to form PM-containing EPFRs [22] and these formed EPFRs have been shown to produce a net output of ROS species in aqueous media [79]. The exact chemical composition and size of the various PM induce different amounts and types of oxidative stress. In lungs of neonatal animals and human bronchoepithelial cell lines (BEAS-2B) exposed to EPFRs, we have observed increased oxidative stress following exposure to combustion generated PM [70, 72]. Indicators of oxidative stress included increases in the ratio of oxidized to reduced glutathione, markers of lipid peroxidation (e.g., 8-isoprostanes), and decreases in antioxidant enzyme activity including superoxide dismutase (SOD), catalase, and glutathione peroxidase. These effects were specifically related to the presence of the EPFR, since size-controlled non-EPFR particles failed to induce significant amounts of oxidative stress either in vivo or in vitro.

The overall role of oxidative stress in the development/exacerbation of lung disease is becoming apparent. Lipid peroxidation indicators such as malondialdehyde have been observed as biomarkers for PM-induced increases in oxidative stress in children with asthma [80]. This is thought to happen as both direct and indirect damage to the lung parenchyma; PM reacts directly with tissue to produce hydroxyl radicals via surface chemistry and indirectly through the actions of inflammatory cell influx. It has also been suggested that the ability to handle oxidative stress is altered in the lungs of asthmatics [81], leading to excessive ROS and its downstream effects (i.e., airway remodeling).

Other studies have shown a relationship between oxidative stress and both innate and adaptive immune function. In particular, ozone (O₃) exposure induces increased cell surface expression of proteins intricately involved in sensing invading pathogens (e.g., toll-like receptor 4 (TLR4)). With O₃ challenge, TLR4 expression increases and is responsible for enhanced immune responses to inhaled allergens in mice [82]. This effect has also been observed in healthy human subjects exposed to O₃ [83, 84]. Oxidative stress also mediates the release of proinflammatory cytokines and increases antigen presentation because of immunosuppressive cytokine interleukin 10 (IL-10) downregulation leading to increased sensitivity and inflammation [85]. The role of oxidative stress and defense from oxidative damage is also an important factor in viral infection as demonstrated by higher viral burden in mice with antioxidant-deficient diets [86] and the attenuation of infection with treatment with the antioxidant glutathione [87].

PM exposure can also lead to changes in the development of adaptive immunity. Diesel exhaust particles, ambient PM, and O₃ have all been shown to act as adjuvants in rodent models of inflammatory airways disease [88,89]. More recent studies have demonstrated the direct effects of PM on mediators of adaptive immunity. With PM exposure, pulmonary dendritic cells (DCs), the professional antigen presenting cells of the immune system, increase in number and activation status [90], whereas T cells demonstrate greater proliferative ability [91]. We have also observed the exacerbating potential of EPFR-containing PM during coexposure with allergen (OVA) in sensitized adults (data not shown). Interestingly, while these adjuvant-like effects of PM foster a heightened inflammatory response in adults, the infant immune system is very different and such heightened inflammatory responses can be quite detrimental in inhibiting adequate respiratory gas exchange and are thus often suppressed.

While our data suggest EPFR-mediated oxidative damage to neonatal lungs, there exist a plethora of data demonstrating that neonates are more resistant to oxidative damage as compared to adults [92, 93]. However, the actual data on this subject are less clear. SOD activity in most strains of mice increases naturally with age and peaks at 25 weeks of age [94], and SOD-2 has been shown to be induced by cytokines including IL-1 β and TNF α both in vitro [95] and in vivo [96] models of hyperoxia; and neonates express these cytokines in higher amounts than adult mice [93]. Cumulatively, these data suggests an “oxidative-resistant” phenotype and that during this unique developmental period, the neonate is better equipped at handling additional oxidative damage *to an extent* because their bodies are primed to prepare for their first encounter with oxygen (i.e., SOD-1 activity is “programmed” to increase shortly after birth and the signals for SOD-2 induction are easily/rapidly produced). This is contrary to adults where the oxidant/antioxidant balance has been well established, and subsequent insults prove detrimental.

Although neonatal mice have been shown to be more resistant to oxidative stress, severe focal alveolitis has been observed in response to hyperoxia indicating that pulmonary injury still occurs and is a major concern given the developmental status of the lung [93]. Consistent with this finding, our data show EPFR-containing PM cause alveolar septal destruction at a much higher degree than non-EPFR PM [70], suggesting some oxidative component.

Infant Immunity: An Age Naturally at Risk

Shortly after birth, the neonatal immune system is bombarded with antigens, particularly at surfaces with high external exposure such as the respiratory tract. Neonates and infants must deal with pathogens using both innate immunity, mediated by pattern recognition receptors including TLRs, and adaptive immunity, mediated by helper and cytotoxic T cells and B cells. As previously stated, the infant immune system is very different from that of the adult.

Innate immunity in the infant is impaired and characterized by selective TLR signaling (i.e., reduced overall TLR expression; responses to TLR8 ligands, and little-to-no responses to TLR1-7 ligands) and reduced ability to produce proinflammatory and Th1 cytokines (TNF α , IFN γ) upon TLR stimulation [97]. In addition, adaptive immunity in the infants is also very different from that of a mature adult. Neonates have impaired Th1 (IFN- γ , IL-12) responses but preserved Th2 responses (IL-4, IL-13) due to epigenetic differences (hypermethylation and hypomethylation, respectively) at cytokine regulatory regions [98–102]. This early bias is thought to be a result, at least in part, of the actions of immature DCs. Neonatal DCs display attenuated recruitment to the lung [103, 104] and a lower production of IL-12 leading to suppressed Th1 responses and resulting in Th2-biased responses [105]. In addition to suppressed Th1 responses, PM has been shown to suppress regulatory T cells (Treg) through hypermethylation of the Treg-transcription factor Foxp3 in the peripheral blood of children with asthma [106]. Our own data suggest that PM exposure in neonates initially causes an enhanced Treg response followed by a suppression of effector T helper (Th1 and Th2) responses to antigen such as influenza or ovalbumin. The implications of this are that early life exposure to PM creates a suppressive immune environment in the lung whereby the infant immune system cannot generate a protective effector T cell response to certain antigens/pathogens, resulting in enhanced disease. Since adaptive immunity relies on this initial response to “adapt,” reexposure to the same antigen at a subsequent time point invokes an altered, more heterogeneous immune response that is also less protective (Figure 4). Whether this phenomena is PM composition-specific remains to be seen.

Size of PM Is Important in Predicting Respiratory Impact

We have previously reported that in a human laryngeal epithelial cell line, EPFR-containing particles 2.5 μm in diameter (EPFR_{2.5}) are able to continuously generate H₂O₂, while modifying the activities of antioxidant enzymes associated with the neutralization of H₂O₂ (i.e., catalase) [76]. No significant change in the activity of SOD was observed suggesting that O₂^{•−} is not generated in exposed cells. The significant generation of oxidized phospholipids (i.e., 8-isoprostanes) in the culture supernatants, along with the fact that H₂O₂ readily diffuses across the cell membrane, indicated that EPFR_{2.5} induces the generation of ROS outside of the cell. In vivo studies demonstrated that EPFR_{2.5} induces only limited effects on pulmonary redox balance, increasing SOD activity and decreasing catalase activity, and not altering glutathione peroxidase activity or glutathione balance [76].

In contrast, our data using EPFR-containing particles 0.2 μm in diameter (EPFR_{0.2}) have shown marked effects including drastic increases in oxidative stress and significant airway remodeling [70, 72]. EPFR_{0.2} exposure increased the oxidized: reduced glutathione ratio and the level of the lipid peroxidation marker, 8-isoprostanes, in our neonatal rodent exposure model. In association with oxidative stress, we observed epithelial-to-mesenchymal transitions in vitro in EPFR_{0.2}-exposed primary neonatal epithelial cells and in vivo in EPFR_{0.2}-exposed neonatal mice and rats [72]. With multiple days of exposure, smooth muscle mass underlying the bronchial epithelium was increasingly greater and septal lesioning was evident in the alveoli.

Cumulatively, these data indicate that EPFR_{2.5} generates H₂O₂ in the lungs of exposed neonatal animals whereas EPFR_{0.2} are able to penetrate much deeper into alveoli altering antioxidant capabilities. More interestingly, our data suggest that the pulmonary antioxidant defense system exhibits site-specific activity (i.e., catalase is essential in neutralization of ROS generated in the epithelial cells of the trachea and bronchi, and glutathione peroxidase is the main antioxidant involved in detoxification of oxidants in the alveolar region). This is supported by other published observations [107–109] and further suggests that the impact effect of EPFRs is limited to their site of deposition, which in the neonatal rodent lung means that EPFR_{2.5} affects mainly the terminal bronchioles and EPFR_{0.2} affects the

developing acinar structures. Our results are supported by recent experimental data demonstrating that ultrafine particle deposition occurs in the developing alveoli in neonatal rat lungs [110]. The human infant lung and respiratory system are obviously larger than that of an adult rat/mouse, much less an infant rat/mouse, suggesting that the independent size effects observed in the rodent models will occur simultaneously in the human infant. Various models of age-dependent particulate deposition in different lung compartments have shown that infants are alarmingly twice as susceptible to ultrafine particle deposition compared to adults and that most accumulation occurs in the alveolar region of the lower airways [111].

Barrier Dysfunction and Airway Disease

The respiratory epithelium is the first layer of defense against pollutants and aeroallergens. The protective function of the epithelium is maintained through the expression of proteins that allow for tight cell–cell adhesion (E-cadherin, β -catenin, zona occludens 1–3). Patients with asthma readily display reduced expression of these junctional proteins [112], and confluent cultures of cells from asthmatics display decreased transepithelial resistance, which indicates monolayer permeability and a heightened vulnerability to antigen and possibly pathogen access to the basement membrane [113]. Cigarette smoke extract (which contains EPFR-containing PMs) induces decreased transepithelial resistance and increased permeability to aeroallergens such as house dust mite (HDM) in vitro [114].

Our own research using EPFRs in an acute animal exposure model has demonstrated damaging effects of PM on airway epithelium [72]. Within 4 days of exposure, the cells lining the epithelium appear to be highly proliferative with decreased cell:cell junctions and increased epithelial membrane permeability. In subsequent exposure days, this is accompanied by a significant increase in airway remodeling made evident by massive increases in smooth muscle cell mass and number surrounding the airways (Figure 5).

Interestingly, disruption of the bronchial epithelium also strongly effects epithelial:DC interactions. Such interactions are crucial in dictating DC maturation and the ensuing immune response. At homeostatic baseline, free β -catenin in the cytosol of airway DCs is scarce, since it is either tethered to the cell membrane by E-cadherin [115] (which allows adherence to epithelial cells) or it is targeted for proteolytic degradation [116]. Cytosolic β -catenin is only observed under conditions of disrupted E-cadherin-mediated adhesion of epithelial cells and DCs or when wingless (Wnt) signaling is activated. Accumulation of cytosolic β -catenin eventually results in nuclear translocation and the initiation of various transcription events including *Serpine1* and *Snail1* [115] and is strongly associated with IL-10+ regulatory DCs [117]. Since many aeroallergens, including HDM, display intrinsic protease activity as well as signaling capabilities that can drive epithelial-to-mesenchymal transition [118], it must be noted that the continuous interaction between these two factors could be synergistic in promoting airway dysfunction and remodeling as well as affecting development of adaptive immune responses. This is something that we are vigorously investigating.

CONCLUSION AND PERSPECTIVE

EPFR-PM exposure disproportionately affects infants and children and is the cause of significant global mortality and morbidity. Infants/neonates represent a sensitive life stage with unique PM-induced pulmonary disease. Knowledge of the impact of PM on the protective epithelial layer of the respiratory tract along with how that damage modulates long-term immune responses should lead to a focus on how these responses affect the developing neonatal lung and immune system. While much correlative epidemiological data exist, there is currently little-to-no literature establishing a mechanism in infants. Our studies

have shown that the influence of EPFR-containing PM on the developing epithelium proves to be of great significance in modifying functional physiology as well as immunity. Ultimately, understanding infant susceptibility and how it affects allergic sensitization/asthma will open doors to new treatments and preventative therapeutics and more importantly, help promote and mold new legislative policies aimed at reducing environmental exposures to reduce these *preventable* adverse health consequences in addition to saving millions in healthcare dollars.

PM-induced pulmonary disease is a neglected area of global health that affects a large proportion of the world's population. It has become increasingly clear that properties of the various PM are responsible for the observed health effects. This presents a challenge to researchers due to the extreme variability in content of “real-world” PM samples. Differences in sampling methods, extraction methods, dosing concentrations, exposure models, etc. could prove to be monumentally responsible for the variability seen in experimental results. Combustion, laboratory-generated PM offers some advantages such as increased reproducibility but lacks the full spectra of “ingredients” that may also prove to be vitally important in the initiation of health effects. Looking forward, the most pressing areas of research are

- Improved exposure assessment methodologies:
 - to more accurately assess airborne PM associated toxicants (e.g., EPFRs)
 - to understand how sample collection, extraction, and other downstream treatments alter the chemical species of the sample
 - to assess the effect of “storage time” on the biological activity of the environmental samples
- Additional toxicological studies:
 - to help determine acute and long-term health risks
 - to develop viable intervention therapeutics
 - to identify biomarkers to aid epidemiological studies and determine level of present and past exposure
- Additional epidemiological studies to examine the effect on:
 - infectious disease severity and outcomes
 - growth and development
 - other disease states (e.g., diabetes, cardiovascular disease)

These important issues must be addressed in order for accurate and unbiased conclusions to be drawn from PM research and for the true health impacts of EPFR-particle systems to be elucidated.

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FIGURE 1.

Persistence of EPFRs in mouse BALF. EPFR-containing PM (2-MCP on 3% CuO/silica) were suspended in BALF and shaken continuously. At the indicated times, samples were centrifuged for 5 min; and the liquid fraction discarded and the particle fraction retained. Particles were frozen at -25°C and dried in a vacuum for 12 h at room temperature. The analysis of the EPR analysis of the particles demonstrated the presence of paramagnetic signal with a characteristic g value of 2.0042 and a peak width of ~ 8 Gauss (inset). These spectra were similar to preexposure samples and are characteristic to the presence of EPFRs associated with particles. The position and width of the radical signal was not affected by incubation in BALF; however, the intensity (i.e., the number of radicals) diminished with increasing incubation time.

FIGURE 2.

Pubmed Search Results. (A) Number of publications available on asthma and particulate matter. Search conducted on May 23, 2012. (B). Number of published articles available on influenza and child respiratory and particulate matter. Search conducted on May 21, 2012.

FIGURE 3.

Airways hyperreactivity following neonatal exposure to EPFR-containing PM persists in the adult. Neonatal rodents were exposed to EPFR-containing PM or control air for 7 consecutive days then allowed to mature to 8 weeks of age. Enhanced pause (P_{enh}), a derived measure of airways hyperresponsiveness, was measured with increasing doses of inhaled methacholine using whole body plethysmography. $n = 6-8$ animals/group. * $p < 0.05$ Two-way ANOVA.

FIGURE 4.

Neonatal exposure to EPFR-containing PM causes an early Treg response that dampens subsequent effector T cell responses to antigen. (A) Descriptive overview. (B) Responses observed in our lab using our neonatal PM exposure model. With exposure beginning on day 3 of age, we see an increase in the amount of Tregs present in the lung that peaks around the fifth day of exposure. When the lung is given an additional insult at this time (e.g., allergen, influenza), effector T cell responses are dampened compared to nonexposed controls given the same challenge.

FIGURE 5.

Neonatal acute exposure to EPFR_{0.2} causes airway remodeling. (Top panel) Light micrographs of terminal bronchioles from 15-day old rat lungs exposed to control air, non-EPFR_{0.2} (DCB50), or EPFR_{0.2} (DCB230). Black arrows denote significant bronchiolar associated lymphoid tissue (BALT), and black line denotes significant increases in smooth muscle mass surrounding bronchiole. (Bottom panel) Micrographs of lung sections stained by immunohistochemistry for E-cadherin (green), α -smooth muscle actin (red), and cell nuclei (blue) with quantitative assessment of smooth muscle layer (inset). Figure adapted from Balakrishna et al. [70]. BioMed Central.