Murine Lung Responses to Ambient Particulate Matter: Genomic Analysis and Influence on Airway Hyperresponsiveness

Ting Wang,1,∗ Liliana Moreno-Vinacso,1∗ Yong Huang,2 Gabriel D. Lang,1 Jered D. Linares,1 Sascha N. Goonewardena,1 Alayna Grabavoy,1 Jonathan M. Samet,3 Alison S. Geyh,4 Patrick N. Breyssse,4 Yves A. Lussier,2 Viswanathan Natarajan,1 and Joe G.N. Garcia1

1Section of Pulmonary and Critical Care Medicine, and 2Section of Genetic Medicine, Department of Medicine, University of Chicago, Chicago, Illinois, USA; 3Department of Epidemiology, and 4Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA

BACKGROUND: Asthma is a complex disease characterized by airway hyperresponsiveness (AHR) and chronic airway inflammation. Epidemiologic studies have demonstrated that exposures to environmental factors such as ambient particulate matter (PM), a major air pollutant, contribute to increased asthma prevalence and exacerbations.

OBJECTIVE: We investigated pathophysiologic responses to Baltimore, Maryland, ambient PM (median diameter, 1.78 µm) in a murine model of asthma and attempted to identify PM-specific genomic/molecular signatures.

METHODS: We exposed ovalbumin (OVA)-sensitized A/J mice intratracheally to PM (20 mg/kg), and assayed both AHR and bronchoalveolar lavage (BAL) on days 1, 4, and 7 after PM exposure. Lung gene expression profiling was analyzed in OVA- and PM-challenged mice.

RESULTS: Consistent with this murine model of asthma, we observed significant increases in airway responsiveness in OVA-treated mice, with PM exposure inducing significant changes in AHR in both naive mice and OVA-induced asthmatic mice. PM evoked eosinophil and neutrophil infiltration into airways, elevated BAL protein content, and stimulated secretion of type 1 T helper (Th1) cytokines [interferon-γ, interleukin-6 (IL-6), tumor necrosis factor-α] and Th2 cytokines (IL-4, IL-5, eotaxin) into murine airways. Furthermore, PM consistently induced expression of genes involved in innate immune responses, chemotaxis, and complement system pathways.

CONCLUSION: This study is consistent with emerging epidemiologic evidence and indicates that PM exposure evokes proinflammatory and allergic molecular signatures that may directly contribute to the asthma susceptibility in naive subjects and increased severity in affected asthmatics.

KEY WORDS: airway hyperresponsiveness, asthma, interleukin, particulate matter, toxicogenomics.

Environ Health Perspect 116:1500–1508 (2008). doi:10.1289/ehp.11229 available via http://dx.doi.org/ [Online 20 June 2008]

Extensive epidemiologic research confirms the association between increasing cardiopulmonary morbidity and mortality and short-term exposure to ambient particulate air pollution (Dominici et al. 2003; Samet and Krewski 2007). Although the relative risk estimates are small, public health concerns exist because of the large population under exposure and the existence of high-risk groups, such as the elderly, diabetics, and those with cardiopulmonary diseases (Johnson and Graham 2005). Increase in particulate air pollution levels are associated with increased hospital admissions and emergency department visits for respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD) (Brunekreef and Forberg 2005). Unfortunately, despite significant epidemiologic evidence, experimental studies of animals and human exposure to inhaled concentrated ambient particulate matter (PM) have yet to provide substantial insights into specific mechanisms of the pathophysiologic consequences of exposure to ambient PM (Handzel 2000; Peden 2001).

Preclinical animal models have been used to examine the relationship between exposure to PM and airway pathogenesis and elevated airway hyperresponsiveness (AHR) (Archer et al. 2004; de Haar et al. 2006; Walters et al. 2001) both in naive animals and in animals sensitized by antigen challenge (Fernvik et al. 2002; Steerenberg et al. 2003). However, it remains unclear as to which mechanisms are involved in producing local airway damage and adjuvant activity to antigen challenge. To address potential mechanisms of PM-mediated AHR in both control and at-risk populations, we employed a murine model of AHR and asthma induced by sensitization to ovalbumin (OVA). We exposed naive and OVA-sensitized mice to Baltimore, Maryland, ambient PM, a challenge we previously demonstrated to produce acute airway toxicity (Walters et al. 2001). In addition, we applied genomic strategies to characterize acute PM pulmonary effects in OVA-challenged mice in order to define molecular signatures produced by PM, an approach successfully employed in other inflammatory lung disorders (Girgis et al. 2005; Grigoryev et al. 2004; Ma et al. 2005; Nonas et al. 2007; Simon et al. 2006). We now report that acute ambient PM exposure induces significant changes in AHR, accompanied by eosinophil/neutrophil infiltration and type 1 T helper (T1H1)/T1H2 cytokine secretion in both naive mice and OVA-induced asthmatic mice. These studies indicate that the pathophysiologic effects of PM, validated in an OVA-challenged murine model of asthma, may directly contribute to the asthma susceptibility in naive subjects and increased severity in affected asthmatics.

Materials and Methods

Baltimore PM generation. The collection and characterization of ambient Baltimore PM used in these studies have been previously described (Walters et al. 2001, 2002). Briefly, PM was collected from a sixth floor window in urban Baltimore using a high-volume cyclone collector (theoretical cut point of 0.85 μm aerodynamic diameter) intermittently operated over a period of months with a flow rate of 0.6 m³/min. Collected PM was pooled and refrigerated until use. The count median diameter of PM was 1.78 μm with a geometric standard deviation of 2.21.

Murine model of asthma. Male A/J mice (8–12 weeks of age; Jackson Laboratories, Bar Harbor, ME) were housed in an environmentally controlled animal facility at the University of Chicago for the duration of the experiments. All animal procedures conformed to the principles for laboratory animal research outlined by the Animal Welfare Act (1966) and the National Institutes of Health guidelines for the experimental use of animals (Institute of Laboratory Animal Resources 1996), as well as the common guideline of the...
Lung responses to ambient PM in a murine asthma model

We evaluated chip quality, including RNA degradation, reverse transcription, cRNA synthesis and labeling, hybridization, chip washing and scanning, using GCOs, dChip (Li and Hung Wong 2001), and the Bioconductor Affy package (Bolstad et al. 2003). All RNA samples and chips used in this study passed established quality criteria (data not shown). We calculated the intensities of probe sets using the gcma package of Bioconductor software (R Development Core Team 2005) with GC robust multichip average (GCRMA) normalization (Wu et al. 2004). To identify differentially expressed genes, we conducted two-group comparison using Significance Analysis of Microarrays (SAM) (Chu et al. 2003). We defined “dysregulated genes” as the differentially expressed genes identified when a normal control group was used in a pairwise comparison. Only the probe sets that were present (detected by Affy P-call) in all three replicates of at least one group in the pairwise comparison were used for data analysis. The gene-filtering parameters and results are summarized in Supplemental Material, Table 1 (http://www.ehponline.org/members/2008/11229/suppl.pdf). For probe sets representing the same Entrez Gene [National Center for Biotechnology Information (NCBI) 2008a] or UniGene accession numbers (NCBI 2008c), we included only the probe set with the lowest false detection rate (FDR) or the highest fold changes in the gene list. The microarray data have been submitted to the NCBI’s Gene Expression Omnibus (GEO) Datasets (NCBI 2008b) (GSE9465).

Identification of Gene Ontology (GO) categories enriched with dysregulated genes. The functional profiles were represented by the biological processes in the GO database (Gene Ontology Consortium 2006). We compared the number of dysregulated genes in each GO category with that of all genes in the Affymetrix Mouse Genome 430 2.0 Array to determine the significance of the GO category. We performed the analysis using Onto-Express (the Gene Ontology 2008), with the default selection of statistic method (hypergeometric distribution followed by false discovery rate correction). We uploaded the lists of dysregulated genes into Onto-Express to identify significant GO categories (the FDR-adjusted p-value, q ≤ 0.05 with six or more genes).

Ingenuity pathway analysis (IPA). We then uploaded the dysregulated genes into Ingenuity Pathways Analysis (IPA) software (Ingenuity Systems Inc. 2008). This Web-delivered application makes use of the Ingenuity Pathways Knowledge Base (IPKB) containing a large amount of individually modeled relationships between gene objects (e.g., genes, mRNAs, and proteins) in order to dynamically generate significant regulatory and signaling networks or pathways. The submitted genes that it maps to...
the corresponding gene objects in the IPKB are called “focus genes.” The significance of a canonical pathway is controlled by p-value, which is calculated using the right-tailed (referring to the overrepresented pathway) Fisher exact test for 2 x 2 contingency tables. This is done by comparing the number of “focus” genes that participate in a given pathway, relative to the total number of occurrences of those genes in all pathways stored in the IPKB. The significance threshold of a canonical pathway is set to 1.3, which is derived by –log10 (p-value), with p \leq 0.05.

Assessment of gene expression synergy in PM- and OVA-challenged mice. We employed a “synergy,” which we defined as the presence of the effects by PM plus OVA that was greater than the effects induced by PM or OVA alone and greater than the sum of those individual effects (Gong et al. 2007). We used a synergistic index,

\[ SI = \Delta(OVA + PM)/\Delta(\text{PM} + \Delta OVA), \]

where \( \Delta(OVA + PM) \) was the difference between levels of PBS/PBS and OVA/PM groups, \( \Delta(\text{PM} + \Delta OVA) \) was the difference in levels between PBS/PM and PBS/PBS groups, and \( \Delta OVA \) was the difference in levels between OVA/PBS and PBS/PBS groups. Synergy in physiologic parameters required the concurrence of three criteria: SI > 1, \( \Delta(OVA + PM) \) > mean \( \Delta(\text{PM} + \Delta OVA) \), and \( \Delta(OVA + PM) \) > mean \( \Delta OVA \) (p < 0.05).

**Results**

**PM-induced AHR in control and asthmatic mice.** To assess the contribution of ambient Baltimore PM to murine airway inflammation and asthmatic parameters, we used a well-established murine asthma model and assessed AHR as an indirect parameter of airway bronchoconstriction in response to the endogenous bronchoconstrictor Ach (Levitt and Mitzner 1988). We measured airway pressure changes stimulated by exogenously infused Ach to represent airway responses expressed as APTI, a widely used parameter to quantify AHR (Ewart et al. 1995, 1996; Grinnan et al. 2006). We determined that OVA challenge increased AHR in A/J mice on day 1 (1.9-fold increase) and day 4 (1.7-fold increase). AHR remained elevated on day 7 (1.8-fold increase), but was not statistically significant (Figure 1A). Similarly, PM exposure induced a prominent (4.0-fold) increase in AHR in naive A/J mice (PBS controls) on day 1, which waned over the ensuing week, returning to control levels by day 7. When we evaluated the effect of PM exposure on AHR in OVA-challenged mice with the established asthmatic phenotype, a marked synergy in AHR was maximal on day 4 after PM exposure (4.9-fold increase) (Figure 1A). By day 7, unlike either OVA or PM treatment alone, PM-mediated AHR in OVA-treated mice remained significantly elevated compared with PBS-challenged naive A/J mice (2.1-fold increase).

**PM-induced alveolar protein leakage.** We assessed PM-mediated increases in BAL protein level as an indication of epithelial/endothelial barrier dysfunction and vascular leakage, as well as a key parameter of inflammatory lung injury. PM produced significant increases in the level of BAL protein on days 1 and 4 (4.4- and 2.7-fold increase, respectively) in naive A/J mice, with values declining to baseline levels at day 7 after PM exposure (Figure 1B). As expected, OVA challenge induced a mild increase in BAL protein content on days 1 and 4 (1.3- and 1.7-fold increase, respectively). Similar to PM effects in naive mice, PM-induced increases in BAL protein in OVA-challenged A/J mice on days 1 and 4 (> 2-fold increase), with the increased BAL protein levels returning to basal concentrations on day 7 after PM challenge (Figure 1B).

**PM-induced inflammatory leukocyte infiltration into airways.** PM has been...
Lung responses to ambient PM in a murine asthma model

reported to cause inflammatory leukocyte infiltration into airways and alveoli in various animal models (Dick et al. 2003; Kodavanti et al. 1999; Prahalad et al. 1999; Salvi et al. 2000; Walters et al. 2001). We assessed the effect of Baltimore PM on BAL leukocyte counts in both naive and OVA-challenged asthmatic mice (Figure 2A). PM induced a peak increase in total leukocyte count in BAL fluid on day 1 (2.5-fold increase) and remained significantly elevated on day 7. OVA reliably induced an increase in leukocytes on day 1 (1.6-fold increase), and this induction declined back to control levels by day 7. The combination of PM challenge in OVA mice, however, induced strong synergy in leukocyte infiltration into airways, which peaked on day 1 (Figure 2A) but was still highly increased on day 4 and remained elevated even on day 7.

We next analyzed the differential leukocyte types in the extracted BAL. PM induced significant eosinophil (days 1 and 4) and neutrophil (days 1, 4, and 7) infiltration into BAL without any changes in BAL macrophage counts. In contrast, OVA-sensitized mice exhibited increased numbers of macrophages and eosinophils at all three time points (Figure 2B), consistent with reported findings (Kung et al. 1999; Prahalad et al. 1999; Salvi et al. 2000; Walters et al. 2001). Combined OVA and Baltimore PM exposure was similar to OVA exposure alone except for a marked eosinophil infiltration into the airways on day 4, which was greater than either exposure alone (Figure 2B).

**PM-stimulated Th1/Th2 cytokine secretion.** We next examined the levels of Th1 cytokines (IL-6, IFN-γ, TNF-α) and Th2 cytokines (IL-4, IL-5, eotaxin) in BAL fluid (Figure 3) after PM and OVA challenge. Levels of IL-4 and IL-5 were not significantly altered by direct PM exposure or OVA challenge. In contrast, marked increases in IL-4 and IL-5 were produced by the combined challenge of OVA and PM, results that were maximal at day 1 after PM treatment (35- and 9-fold, respectively) and remained significantly elevated through day 4 (3.1- and 2.1-fold, respectively). Levels of the eosinophil chemoattractant eotaxin are known to be increased during eosinophil infiltration and Th2 type inflammation (Mates and Foster 2003; Pease and Williams 2001). Predictably, and consistent with marked eosinophil recruitment, OVA challenge increased eotaxin levels in BAL (days 1 and 4). Despite only a modest effect on BAL eosinophil content, PM increased eosinophil levels in the BAL of naive mice and was markedly synergistic in OVA-challenged asthmatic mice (days 1 and 4). The effects of PM decreased to baseline on day 7 after PM challenge.

Several types of PM are known to stimulate secretion of the proinflammatory cytokines IL-6 and TNF-α (Dick et al. 2003; Walters et al. 2001). We assayed Th1 cytokines (IFN-γ, IL-6, TNF-α) in BAL fluid and discovered a strong PM-mediated Th1-type inflammatory profile with active induction of IL-6 and TNF-α (days 1 and 4) with a minimal effect on IFN except on day 4 (Figure 3D–F). OVA challenge did not affect Th1 cytokine secretion at any time point, and we identified no synergism between PM and OVA that elevated these Th1 inflammatory cytokines.

**PM-induced histologic alterations.** We next confirmed the inflammatory changes observed in lung tissue sections subjected to H&E staining after PM challenge in naive and OVA-challenged mice (Figure 4). We observed inflammatory leukocyte infiltration into vessels, airways, and alveoli after OVA or PM treatment on day 1 after treatment (Figure 4B–D). PM also induced leukocyte infiltration in OVA-challenged asthmatic mice. By day 7 (Figure 4E–H), the amount of infiltrated cells declined in PM-treated mice (Figure 4F,H), consistent with the BAL cellularity analysis (Figure 2A) and cytokine profiles (Figure 3). On day 1, we observed conjugated PM pellets deposited in alveoli, reflecting PM exposure (Figure 4B), but they were gradually eliminated from the lung and became undetectable after 7 days (data not shown). PM also induced mucus secretion into the airways (Figure 5). PM, as well as OVA, activated mucus-producing goblet cells in murine airways day 4 post-challenge. PM exhibited a stronger impact on mucus activation in the OVA-remodeled airways. Although leukocyte infiltration declined after the inhalation of PM, elevated mucus secretion persisted until day 7 (data not shown).

**Differential lung gene expression by PM or OVA challenge.** We next used the Affymetrix platform (Mouse Genome 430 2.0 Array) to profile changes in gene expression after the intratracheal installation of Baltimore PM in naive and OVA-challenged mice. PM had a strong impact on the global expression of lung...
genes, with > 1,200 genes differentially regulated by PM ($p < 0.05$) on day 4 after exposure. With extremely stringent conditions (FDR < 0.3% and fold change > 3-fold), 436 genes survived filtering and were identified as significantly dysregulated by PM exposure (375 genes up-regulated and 61 genes down-regulated). In contrast, OVA challenge had less impact on lung gene expression than did PM at day 4 after exposure. Only 37 genes (21 genes up-regulated and 16 genes down-regulated) were differentially regulated by OVA sensitization even when less stringent criteria (FDR < 5% and fold change > 2-fold) were applied. The combination of PM and OVA treatment exhibited synergistic effects on lung gene expression, with a total of 591 genes identified as differentially regulated [492 genes up-regulated and 99 genes down-regulated (FDR < 0.3% and fold change > 3-fold)].

The PM-regulated genes were related to 22 biological processes, including innate immune response, chemotaxis, cell-surface receptor-linked signal transduction, inflammatory response, defense response, cell cycle, nervous system development, and DNA-dependent regulation of transcription (Table 1). Of the 436 PM-regulated genes, we defined 25 genes as the most differentially regulated (fold change > 25-fold; Supplemental Material Table 1 available online at http://www.ehponline.org/members/2008/11229/suppl.pdf). Interestingly, 17 of these 25 genes were closely linked with asthma/airway inflammation, immune responses, chemokine/cytokine, inflammation, and epithelial cell proliferation (Supplemental Material, Table 2 available online at http://www.ehponline.org/members/2008/11229/suppl.pdf). These data indicate that PM produces profound lung inflammation in a manner that contributes to asthma phenotypes, such as inflammatory leukocytes maturation, cytokine secretion, and airway remodeling.

IPA analyzes the pathways generated by differentially expressed genes in a pairwise fashion compared with either lung gene dysregulation with PM or the combined PM and OVA challenge (Figure 6). Similar to GO analysis, cell cycle, inflammatory response (interleukin signaling, IFN signaling), and cell-surface receptor (B-cell receptor, T-cell receptor, and Toll-like receptor) pathways were among the most distinctly regulated pathways. Most of these signaling pathways are closely related to asthma development. For example, the genes in the complement system were significantly regulated by PM in both the control (PM group) and the asthma animals (PM and OVA group). These genes are implicated in the development of asthmatic phenotypes (Wills-Karp 2007).

**Regulation of asthmatic genes by PM.** The expression profiles of the most differentially regulated asthma genes (by OVA) across all samples were next displayed in dChip (Figure 7). Most of the OVA-regulated genes were consistent with previously reported data (Izuhara and Saito 2006; Kuperman et al. 2005). Hierarchical clustering on individual samples correctly classified replicates into the corresponding experimental group, indicating that the OVA-regulated genes displayed a differential expression profile in each experimental condition (PM, OVA, PM/OVA). We also
Ambient particles are classified for health purposes by aerodynamic diameter, as follows: coarse [< 10 µm (PM10)], fine (< 2.5 µm (PM2.5)), and ultrafine/nanoparticles [< 0.1 µm (PM0.1)]. We used a single Baltimore PM sample with a median diameter of 1.78 µm, which allows adequate upper and lower respiratory tract deposition (Figure 4B, D) when delivered via intratracheal instillation (Walters et al. 2001, 2002). This intratracheal administration mode is not representative of physiologic conditions and represents a high dose rate. This single administered dose is equivalent to an exposure over a period of months. We recognize potential limitations with this approach.

A key finding in the present study is the demonstration that PM not only induces AHR in naive mice but also is synergistic in...
exacerbating AHR in mice with preexisting enhanced AHR (OVA-sensitized mice 4 days after PM exposure). Because diesel exhaust particles, a major source of Baltimore PM, are recognized as adjuvants during allergen exposure (Nel et al. 1998; Ohta et al. 1999; Porter et al. 2007; Walters et al. 2001), the possibility that ambient PM is generally involved in the increased prevalence of human atopic asthma is highly plausible. We did not directly address the hypothesis that PM contributes to asthma susceptibility; however, our data collected from mice with established increased AHR indicate that PM significantly increases the severity and duration of several features observed during acute asthma exacerbations (Walters et al. 2001). The findings include increased BAL eosinophils and BAL eotaxin and IL-5 generation, suggesting that PM directly affects AHR independently of an effect on allergic sensitization. In addition to increased AHR, we observed that PM exposure induces increases in BAL protein, reflecting increases in vascular and epithelial permeability, a cardinal feature of the inflammatory response likely related to elevated levels of PM-containing reactive oxygen species (Gualtieri et al. 2007; Li et al. 2003) or to PM-mediated recruitment of leukocytes to challenged airways.

Limited information exists concerning the toxicogenomic effects of PM on lung tissue, a viable strategy for the identification of potential molecular biomarkers that may reflect the pulmonary toxicity of PM exposure (Andre et al. 2006; Koike et al. 2004; Kooter et al. 2005; Leikauf et al. 2001; Roberts et al. 2004; Wise et al. 2006). Because asthmalike parameters peaked 3–7 days after PM treatment (Walters et al. 2001), we chose day 4 after PM exposure as the time point to assess the impact of PM on lung tissue genome regulation, a time point of maximal synergy with OVA sensitization. The PM impact on global gene expression was extremely strong, despite extremely stringent conditions, with most of the 436 filtered genes representing biological processes closely associated with asthmatic parameters, such as immune responses, innate immune responses, inflammatory responses, and leukocyte chemotaxis. Although ambient particle-induced oxidative stress may contribute to inflammatory and toxic effects (Li and Nel 2006; MacNee and Donaldson 2003; Xia et al. 2007), we did not identify this gene ontology within the 436 genes in the SAM list.

Our genomic results (confirmed by enzyme-linked immunosorbent assay and reverse-transcriptase polymerase chain reaction approaches) show the previously described propensity for PM to induce a strong pro-inflammatory molecular signature, as well as a strong genomic signature involving activation of biological pathways linked to the development of asthmatic phenotypes, such as the induction of the modulation and secretion of key T112 cytokines (e.g., IL-4, IL-5, eotaxin). Eosinophilic inflammation is a hallmark of asthma, and exposure to Baltimore PM was a potent stimulus for an influx of eosinophils (as well as neutrophils) into the murine lung (Figures 1B, 2, 4) and synergistically induced eosinophil infiltration in asthmatic mice, likely major contributors to the sustained AHR response in PM-stimulated OVA-sensitized mice. IL-4, a cytokine with marked PM-mediated increases in gene and protein expression, regulates allergic inflammation by eosinophil adhesion and recruitment to lung airways, promotes T112 cell differentiation, and directly stimulates airway remodeling (Kips 2001). Both IL-5, a primary cytokine involved in eosinophil differentiation, maturation, and

---

**Figure 6.** Biological processes detected by IPA of OVA/PM and PBS/PM dysregulated genes. Abbreviations: NF-κB, nuclear factor-κB; PPAR, peroxisome proliferator-activated receptor. Several of the top significant canonical pathways were enriched with dysregulated genes induced by PM or OVA/PM treatment. The horizontal line indicates the threshold. See Supplemental Material, Table 2 (online at http://www.ehponline.org/members/2008/11229/suppl.pdf) for the description of gene selections with SAM software.

**Figure 7.** Hierarchical clustering of OVA-induced dysregulated genes by dChip. The 37 dysregulated genes induced by OVA treatment were selected by SAM software (see Supplemental Material, Table 1, online at http://www.ehponline.org/members/2008/11229/suppl.pdf). Sample clustering is displayed at the top; the two gene clusters A and B are displayed on the left. Blue, white, and red represent the expression level below, at, and above mean level, respectively. PBS1, PBS control sample 1; PM1, PM-treated sample 1; OVA1, OVA-treated sample 1; OVAPM1, OVA- and PM-treated sample 1.
activation (Hogan et al. 1998), and eotaxin, an eosinophil chemotactant (Conroy and Williams 2001), exhibited markedly up-regulated expression by PM in asthmatic mice. These results provide a viable mechanism for PM contribution to asthma development and propensity for asthmatic exacerbations.

PM-mediated gene expression overlaps significantly with many OVA-driven genes identified in our model of murine asthma with expression in exactly the same direction (often with significant synergy); that is, PM downregulates all OVA-down-regulated genes and up-regulates all OVA-up-regulated genes (Figure 7). OVA induces 37 differentially expressed genes, with 14 of these genes also listed within the PM-regulated SAM list (Table 2) including the potent asthma biomarkers Clea3 and Tff2 (Nikolaidis et al. 2006). Clea3 encodes a calcium-activated chloride channel found to be critical in mucus overproduction and AHR in asthma (Nakanishi et al. 2001). The Tff2 cytokines IL-4 and IL-13 induce expression in epithelium in association with goblet cell metaplasia, mucus overproduction, and augmented allergic airway responses (Berlin et al. 2004). Tgfβ factor-2 (Tff2) is an allergen-induced gene regulated by Tff2 cytokines in the lung that promotes human bronchial epithelial cell migration and formation of mucus-producing airway cells (Nikolaidis et al. 2006). Consistent with the dramatic up-regulation of genes that encode factors that affect goblet cell activity and mucus production, such as Tgfβ, Clea3, and Muc5b, another marker for mucus stimulation, PM exhibited prominent effects on mucus stimulation as indicated by periodic acid-Schiff (PAS) staining (Figure 5) in both control and OVA-challenged mice.

Analysis of the gene ontologies stimulated by PM in naive mice demonstrated similarities to prior PM-related genomic studies with the induction of airway inflammation activity. For example, acute exposure (4–24 hr) of ultrafine PM induced expression of genes involved in oxidation stress, inflammation, transcription regulation, and cardiovascular-related function without a link to asthma development and exacerbation (Andre et al. 2006; Kooter et al. 2005). IL-6 and TNF-α are proinflammatory cytokines that regulate innate immunity physiologic host defense processes during infection, airway damage in COPD, and acute lung injury (Chung 2001). Unlike results in OVA-challenged mice alone, PM stimulates the generation and secretion of IL-6 and TNF-α into airways (Figure 4) with marked neutrophil infiltration. Another key PM signature in naive mice is involvement of complement activation pathways. Complement factors C3 and C5 are potent chemotactants for inflammatory cells (neutrophils and eosinophils) and anaphylatoxins, which trigger smooth muscle contraction and regulate vasodilation (Bolger et al. 2007). C3 and C5 are also known to increase vascular permeability, a clear phenotypic parameter in PM-challenged OVA mice where PM exposure induces significant changes in vascular permeability as assessed by protein content in BAL fluids (Figure 2A). Complement factor B is a key regulator in the development of AHR and inflammation (Taube et al. 2006). In addition, we identified complement factor 3 to mediate PM-induced AHR, indicating that AHR induced by PM depends on C3 activation in the airways (Walters et al. 2002). Consistent with a role in mediating PM-regulated airway function, we found five genes from the complement system family that are differentially affected by PM: complement component 1, q subcomponent, alpha polypeptide (3.0-fold); complement component 1, q subcomponent, beta polypeptide (3.2-fold); complement component 1, q subcomponent, beta polypeptide (3.2-fold); complement component 1, q subcomponent, C chain complement (3.5-fold); component 3a receptor 1 (35-fold); and complement factor B (17-fold).

Our genomic approaches also allowed us to identify multiple, potentially novel, biomarkers for development of asthmatic lung responses induced by PM. Rgs9 is a family member of regulators of G-protein signaling that act as GTase-activating–proteins specific to the G protein subunit. It plays a critical role in the termination process of G-protein–mediated cell responses in eukaryotes (Hepler 1999). Rgs9 in retinal photoreceptor cells induces an elevation in local cyclic guanosine monophosphate (cGMP) concentration and closes cGMP-gated cation channels (Arshavsky et al. 2002). PM reduced Rgs9 transcription in lung tissues (6.2-fold), whereas OVA had a similar effect (5.2-fold). Our finding that Rgs9 is highly transcribed in lung tissues is novel, although a localized function has not yet been elucidated. Intelectin is an intestinal antimicrobial factor (Datta et al. 2005) induced by IL-13 in human airway epithelial cells (Kuperman et al. 2005). The molecular patterns recognized by intelectin include furosides such as galactofuranose and galactofuranosyl residues that are present in bacterial and fungal cell walls and in protozoan parasites, but not in mammalian cells. Intelectin was markedly induced by PM (35-fold increase) and represents another potential signature of PM-induced lung toxicity. Although intelectin in the airway may serve as a novel defensive gene altering the response of asthmatics to infection, the contribution of intelectin to asthma pathogenesis requires further exploration.

In summary, exposure of a murine preclinical model of asthma to urban PM results in elevation of AHR, BAL protein leakage, inflammatory leukocyte infiltration, and Tgfβ cytokine secretion. Baltimore PM also produced a strong molecular signature composed of inflammatory/asthmatic gene expression in naive mice, with marked synergy with OVA-sensitized mice. These studies are consistent with emerging epidemiologic evidence and indicate that PM exposure evokes proinflammatory and allergic molecular signatures that may directly contribute to the asthma susceptibility in naive subjects and increased severity in affected asthmatics.

### Table 2. Intersection between PM- and OVA-induced dysregulated genes.

| Probe set ID | Symbol | OVA | PM | GO category |
|-------------|--------|-----|----|-------------|
| 1439423_x_at | AA68098 | 2.8 | 3.2 | Lipid binding |
| 1419684_at | Ccl8 | 26.2 | 26.7 | Chemotaxin |
| 1416306_at | Clea3 | 3989.6 | 64.0 | Chloride transport |
| 1434046_at | AA467197 | 4.6 | 68.8 | Other |
| 1459303_at | Isl1 | 0.3 | 0.2 | Cell differentiation |
| 1447197_x_at | LOC207885 | 7.4 | 3.2 | Humoral immune response |
| 1418165_at | Itlna | 197.5 | 35.3 | Signal transduction |
| 1439635_at | Rgs9 | 0.2 | 0.2 | G-protein coupled receptor protein signaling pathway |
| 1449015_at | Retnla | 16.2 | 13.4 | Hormone activity |
| 1422040_at | Sema7a | 0.3 | 0.3 | Multicellular organismal development |
| 1454409_at | S100a16 | 3.8 | 3.9 | Protein amino acid glycosylation |
| 1443381_at | Tspan9 | 0.3 | 0.2 | Other |
| 1422448_at | Thf2 | 20.3 | 12.3 | Other |
| 1455114_at | Ug2 | 2.2 | 11.8 | Regulation of progression through cell cycle DNA repair |

Fold changes of dysregulated genes selected by SAM software (Supplemental Material Table 2, online at http://www.epiethonline.org/members/2008/11228/suppl.pdf). For all genes, q < 0.1%. Corresponding GenBank accession numbers are available from Affymetrix (https://www.affymetrix.com/analysis/netaffx/quickquery.affx/metaffx/netaffx4_annot) with probe ID.
effects of coarse airborne particles on health. Eur Respir J 26(2):309–318.

Chu G, Narasimhan B, Ting TH, et al. 2007. Single-nucleotide polymorphisms in the EGF gene, intrauterine growth restriction, and low birth weight. Human Genet 121(6):778–785. Wickham JD, Studer D, et al. 2006. Effect of maternal obesity on offspring airway development and response to allergen. J Allergy Clin Immunol 118(5):1124–1241.

Guattari M, Mantegna R, Cattuto C, et al. 2003. Ultraviolet radiation in the atmosphere is a major source of DNA damage in human cells. Proc Natl Acad Sci USA 100(6):3349–3354.

Handzel ZT, Young JM, Rothenberg ME, Ramsay AJ. 2001. DNA microarray analysis of rat alveolar epithelial cells following exposure to organic dye extract of diesel exhaust particles. Toxicol Appl Pharmacol 172(2):178–185.

Kuo I, Oppenheim JJ, Casale TF. 2005. Gene expression pattern in spontaneously hypertensive rats exposed to urban particulate matter (EHC-93). Inhal Toxicol 17(1):53–65.

Kung TT, Jones H, Adams GE III, Spengler JD, Egan RW, et al. 1994. Characterization of a murine model of allergic pulmonary inflammation. Int Arch Allergy Immunol 103(1–3):183–90.

Kuperman DA, Lewis CC, Woodrow PG, Rodriguez MW, Yang YH, Dolgova GM, et al. 2005. Dissecting asthma using focused transgenic modeling and functional genomics. J Allergy Clin Immunol 116(2):305–311.

Lambert AL, Dietmeier K, Dijkstra DJ, Gilmour MI. 1999. Residual oil ash exposure enhances allergic sensitization to house dust mite. Toxicol Appl Pharmacol 158(3):269–277.

Li C, Yang H, Wang X, et al. 2004. cDNA microarray analysis of rat alveolar epithelial cells following exposure to organic dye extract of diesel exhaust particles. Toxicol Appl Pharmacol 198(1):81–89.

Mitra BR, Broadwell SE, Wesselkamper SC, Miller CR, Hardie WD, Gammom K, et al. 2001. Pathogenetic mechanisms for particulate matter induction of acute lung injury and mortality in mice. Respir Res 2:21.

Mitra BR, Gammom K, et al. 2001. Pathogenetic mechanisms for particulate matter induction of acute lung injury and mortality in mice. Respir Res 2:21.

Mitra BR, Gammom K, et al. 2001. Pathogenetic mechanisms for particulate matter induction of acute lung injury and mortality in mice. Respir Res 2:21.

Mitra BR, Gammom K, et al. 2001. Pathogenetic mechanisms for particulate matter induction of acute lung injury and mortality in mice. Respir Res 2:21.

Mitra BR, Gammom K, et al. 2001. Pathogenetic mechanisms for particulate matter induction of acute lung injury and mortality in mice. Respir Res 2:21.

Nakajima H, Iwamoto I, Tomoe S, Matsumura R, Tomioka H, Kato T, et al. 2001. Role of gob-5 in mucus overproduction and airway obstruction in a mouse model of Kartagener syndrome. Am J Respir Cell Mol Biol 25(4):583–591.

Nakajima H, Iwamoto I, Tomoe S, Matsumura R, Tomioka H, Kato T, et al. 2001. Role of gob-5 in mucus overproduction and airway obstruction in a mouse model of Kartagener syndrome. Am J Respir Cell Mol Biol 25(4):583–591.

Nakajima H, Iwamoto I, Tomoe S, Matsumura R, Tomioka H, Kato T, et al. 2001. Role of gob-5 in mucus overproduction and airway obstruction in a mouse model of Kartagener syndrome. Am J Respir Cell Mol Biol 25(4):583–591.

Nakajima H, Iwamoto I, Tomoe S, Matsumura R, Tomioka H, Kato T, et al. 2001. Role of gob-5 in mucus overproduction and airway obstruction in a mouse model of Kartagener syndrome. Am J Respir Cell Mol Biol 25(4):583–591.

Nakajima H, Iwamoto I, Tomoe S, Matsumura R, Tomioka H, Kato T, et al. 2001. Role of gob-5 in mucus overproduction and airway obstruction in a mouse model of Kartagener syndrome. Am J Respir Cell Mol Biol 25(4):583–591.

Nakajima H, Iwamoto I, Tomoe S, Matsumura R, Tomioka H, Kato T, et al. 2001. Role of gob-5 in mucus overproduction and airway obstruction in a mouse model of Kartagener syndrome. Am J Respir Cell Mol Biol 25(4):583–591.

Nakajima H, Iwamoto I, Tomoe S, Matsumura R, Tomioka H, Kato T, et al. 2001. Role of gob-5 in mucus overproduction and airway obstruction in a mouse model of Kartagener syndrome. Am J Respir Cell Mol Biol 25(4):583–591.