Retraction: The authors have requested that this paper be retracted because of unreliable results in Figures 2, 4, and 5. The authors were unable to repeat the studies reported in Figures 2 and 4 owing to lack of materials. The authors apologize to the *Journal* and to its readers.
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Maternal Exposure to Particulate Matter Increases Postnatal Ozone-induced Airway Hyperreactivity in Juvenile Mice

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***Rationale*: Epidemiologic studies implicate air pollutant exposure during pregnancy as a risk factor for wheezing in offspring. Ozone exposure is linked to exacerbations of wheezing in children.**
***Objectives*: To determine if maternal pulmonary exposure to traffic-related particles during pregnancy augments ozone–induced airway hyperresponsiveness in offspring.**
***Methods*: C57BL6 time-mated mice were given NIST SRM#1648 (particulate matter [PM]) 0.48 mg, saline vehicle, or no treatment by tracheal insufflation twice weekly for 3 weeks. PM exposure augmented maternal lung inflammation and placental TNF-α, Keratinocyte-derived cytokine (KC), and IL-6 (measured at gestation Day 18). After parturition, dams and litters were exposed to air or ozone 1 ppm 3 h/d, every other day, thrice weekly for 4 weeks. Respiratory system resistance in pups was measured at baseline and after administration of nebulized methacholine.**
***Measurements and Main Results*: Ozone increased airway hyperresponsiveness, but the increase was greatest in pups born to PM-treated dams. Whole-lung TNF-α, IL-1β, KC, IL-6, and MCP-1 were increased in ozone–treated pups, with the greatest increase in pups born to dams given PM. Airway epithelial mucous metaplasia estimated by periodic acid-Schiff Alcian blue staining was increased in ozone–exposed pups born to PM-treated dams. Alveolar development, determined by morphometry, and airway smooth muscle bulk, estimated using α-actin histochemistry, were unaffected by prenatal or postnatal treatment.**
***Conclusions*: Maternal pulmonary exposure to PM during pregnancy augments placental cytokine expression and postnatal ozone–induced pulmonary inflammatory cytokine responses and ozone–induced airway hyperresponsiveness without altering airway structure.**

Fetal and neonatal origins of life-long chronic diseases, including lung diseases, have come under increased scrutiny since epidemiologic evidence has linked a number of maternal exposures to increased risk for childhood asthma (1). Maternal exposure to airborne pollutants has been associated with adverse health effects in offspring, including growth restriction (2) and asthma (3, 4). Traffic-related air pollutants that exhibit this linkage include carbon monoxide, particulate matter, nitrogen oxides, and ozone (5). These industrial and traffic-related exposures have been linked epidemiologically to asthma exacerbations in children and may account for some of the observed disparities in respiratory health among children of differing ethnic/socioeconomic backgrounds (6).

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Maternal pulmonary exposure to air pollutants augments antigen-induced airway hyperresponsiveness in juvenile mice. Interaction with ozone-induced hyperresponsivity has not been tested.

What This Study Adds to the Field

Maternal exposure of mice to particulate matter augments placental inflammatory cytokine responses and ozone-induced lung inflammation and airway hyperreactivity in offspring.

Interaction between maternal exposures to airborne pollutants and childhood asthma has been suggested by epidemiologic studies (7). These studies are limited by complex, multiple exposures, the effects of which are difficult to interpret. Until recently, most animal studies have been limited by the application of relatively oversimplified exposure schemes. In addition, comparatively few have addressed neonatal exposures, although recent evidence suggests developmental vulnerabilities to inhaled ultrafine particulate matter (PM) (8).

Recent studies aimed at breaching these gaps have used mouse models of prenatal pollutant exposure combined with postnatal challenges. Maternal pulmonary exposure to diesel exhaust particles late in pregnancy induced changes in mechanics of the respiratory system suggestive of increased sensitivity to methacholine stimulation in response to ovalbumin sensitization/challenge in offspring (9), with parallel findings after maternal exposure to residual oil fly ash (10). Using methods that distinguish small airway from total respiratory system resistance, we and others have demonstrated that exposure to ozone at concentrations relevant to human health increased airway hyperresponsiveness (AHR) in adult mice (11).

We hypothesized that maternal pulmonary exposure to traffic-related pollutant particles during pregnancy could act as a priming event, exacerbating ozone–induced AHR in offspring exposed to ozone during postnatal lung development. Ozone exposure in children has been linked with asthma exacerbations even at levels previously thought to be safe (12–14). As a first step, we used a well-characterized urban PM (SRM#1648) to provoke maternal pulmonary inflammation by tracheal insufflation twice weekly during pregnancy to determine its effects on ozone–induced AHR in newborn mice. We chose a postnatal ozone exposure regimen relevant to human exposures in urban areas during summer (~0.2–0.6 ppm) given the lower deposition fraction in rodents (15).

We found that ozone exposure induced AHR in response to nebulized methacholine challenge in juvenile mice. Maternal PM instillation provoked acute maternal lung inflammation,

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increased fetal placental cytokine expression, and augmented the ozone exposure–induced pulmonary proinflammatory cytokine expression and induction of AHR in mice at 4 weeks postnatal age. Some of the results in these studies have previously been reported in the form of an abstract (16).

METHODS

Animal Exposures

All procedures were approved by the institutional animal use committee. C57BL/6 mice were bred from colony stock (Jackson Laboratories, Bar Harbor, ME) and maintained in vivarium Horsfall isolation units. Time-mated dams were lightly anesthetized with 0.5% isoflurane in a chamber and suspended by the maxillary incisors on a tilt board. Urban particle (see the online supplement for details) (PM) 0.48 or 0.96 mg or saline vehicle (0.9% NaCl) was administered (0.03 ml per mouse; average body weight, 21 g) by orotracheal insufflation as previously described (17). Insufflations were performed twice weekly for 3 weeks (*see* online supplement). Spontaneously delivered dams and litters were placed in wire cages and exposed to ozone 1 ppm for 3 hours three times a week for 4 weeks as previously described (18).

Maternal Bronchoalveolar Lavage Cytology

In three dams per treatment group, animals were killed with sodium pentobarbital (200 mg/kg intraperitoneally) 3 days after a single insufflation (PM or vehicle), and bronchoalveolar lavage was performed with 0.5 ml × 4 washes. Washes were pooled, and cells were counted using a hemacytometer. Cells were centrifuged, stained, and counted (19).

Placental Cytokine Measurements

At gestation Day 18, dams were killed with sodium pentobarbital (200 mg/kg intraperitoneally), and fetuses were rapidly removed and placed on ice. Placentas from two pups in three litters (n = 6) from each treatment condition (air, saline, and PM insufflations) were removed, snap frozen, and homogenized later for IL-1β, TNF-α, IL-6, and Keratinocyte-derived cytokine (KC) cytokine analysis using a multiplex bead-based assay according to the manufacturer's directions (Multiplex; Millipore, Billerica, MA). Protein lysates (200 μg/well) were incubated with antibody beads at 4°C overnight.

Survival, Weight Gain

Body weights for pups were measured every other day, and litters were inspected twice daily for survival.

Measurement of Postnatal Lung Mechanics

At 28 days, pups were anesthetized, and a tracheal cannula was placed before connection to a small animal ventilator equipped with a nebulizer (flexiVent; SciREQ, Montreal, PQ, Canada). Respiratory mechanics measurements were performed (*see* the online supplement for details). Baseline and nebulized methacholine-induced respiratory system resistance was measured at low and high frequencies to distinguish total and large airway (Newtonian) resistance (20).

Pulmonary Inflammation

Lung sections from four juvenile mice per treatment group were stained with hematoxylin and eosin and immunolabeled with antimyeloperoxidase (see online supplement). Proinflammatory cytokines were measured in whole lung homogenates. Specimens were from pups that did not have pulmonary mechanics performed. Protein extracts were quantified using the Bradford method (21), and 1 mg per lung from four to six pups per treatment group was analyzed in duplicate for IL-1β, IL-6, KC, MCP-1, and TNF-α as described above.

Airway Structure

Airway smooth muscle bulk was estimated by selecting random images as noted above from sections immunostained with rat monoclonal anti–α-smooth muscle actin (Epitomics, Burlingame CA), biotinylated secondary antibody, and avidin-peroxidase complex (ABC Elite; Vector, Burlingame CA) with diaminobenzidine substrate after citrate antigen retrieval (Vector). Sections were counterstained with hematoxlin. Airway epithelial mucous metaplasia was scored semiquantitatively by periodic-Schiff/Alcian blue (PAS) as previously described (22). Small airway remodeling was assessed using Masson's trichrome stain.

Alveolar Development

Four random sections per lung from four pups per group were stained with malachite green and Hart's elastin as previously described (21). Alveolar volume density, which estimates alveolar number, and alveolar surface density, which estimates alveolar surface area, were measured as described in the online supplement.

Statistical Analysis

Significant between-group differences were identified by ANOVA with *post hoc* analysis using Tukey's HSD test. Survival analysis was performed using the Kaplan-Meier test using SPSS version 14.

RESULTS

Effect of Maternal PM Exposure on Maternal Lung Inflammation

Both PM doses induced similar increases in BAL leukocytes and similar shifts toward neutrophil influx (Figure 1). The lower dose (0.48 mg per mouse) was used in all of the subsequent experimental exposures.

Effect of Maternal PM Exposure on Placental Cytokine Expression

PM instillation significantly increased placental TNF-α, IL-6, and KC levels compared with saline-instilled and air-exposed control mice (Figure 2). In particular, KC levels were over threefold higher, and IL-6 over two-fold higher, than in control mice. IL-1β was unaffected by maternal saline or PM instillation.

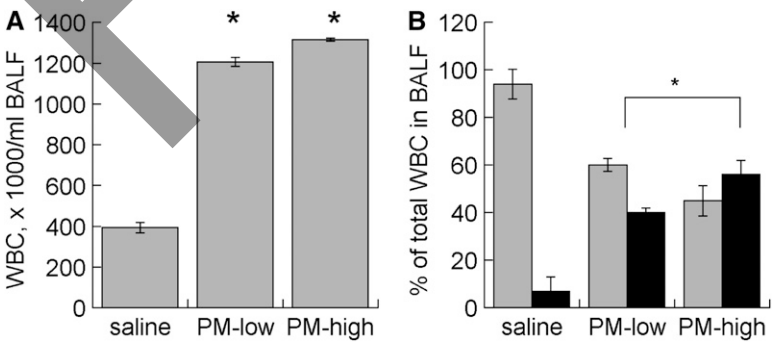

Figure 1. Bronchoalveolar lavage fluid (BALF) (*A*) leukocyte count and (*B*) differential from pregnant mice 24 hours after treatment with low (0.48 mg) or high (0.96 mg) doses of St. Louis particle suspension by orotracheal instillation. Data are mean ± SEM. *$P < 0.05$ vs. saline. PM = particulate matter; WBC = white blood cell count. *Gray bars*, macrophages; *black bars*, neutrophils.

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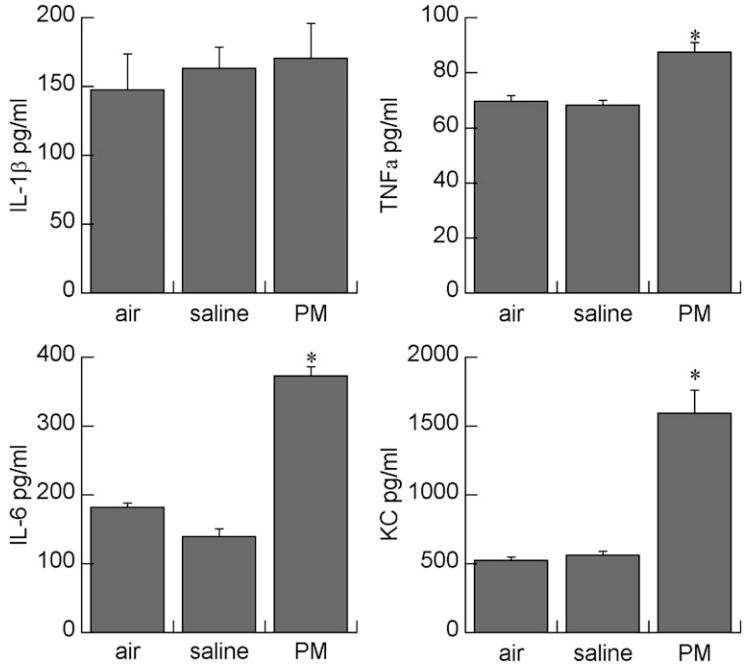

Figure 2. Effects of maternal treatment on placental cytokines. Data are mean ± SEM; n = 6 per treatment group. *$P < 0.001$ vs. saline or air. PM = particulate matter.

Effect of Postnatal Ozone Exposure with or without Maternal PM Exposure on Postnatal Growth

There were no significant effects ($P > 0.1$) of any prenatal or postnatal treatment on postnatal survival, which was greater than 90% in all groups. All treatments (maternal and postnatal) significantly impaired body weight at postnatal Day 8 ($P < 0.001$), with the most significant effects in occurring pups born to saline-treated dams and reared in air and in pups born to PM-treated dams and treated postnatally with ozone (Figure 3). By postnatal Day 28, these differences had narrowed and were no longer significant, with the exception of pups born to PM-treated dams that were exposed to ozone ($P < 0.001$ vs. air).

Effect of Postnatal Ozone Exposure with or without Maternal PM Exposure on Postnatal Lung Inflammation

Postnatal ozone exposure induced increased proinflammatory cytokine levels (IL-1β, TNF-α, KC, IL-6, and MCP-1) in whole lung (Figure 4) compared with postnatal air exposed groups. Ozone exposure increased all measured proinflammatory cytokine levels (normalized to the total protein), which were further

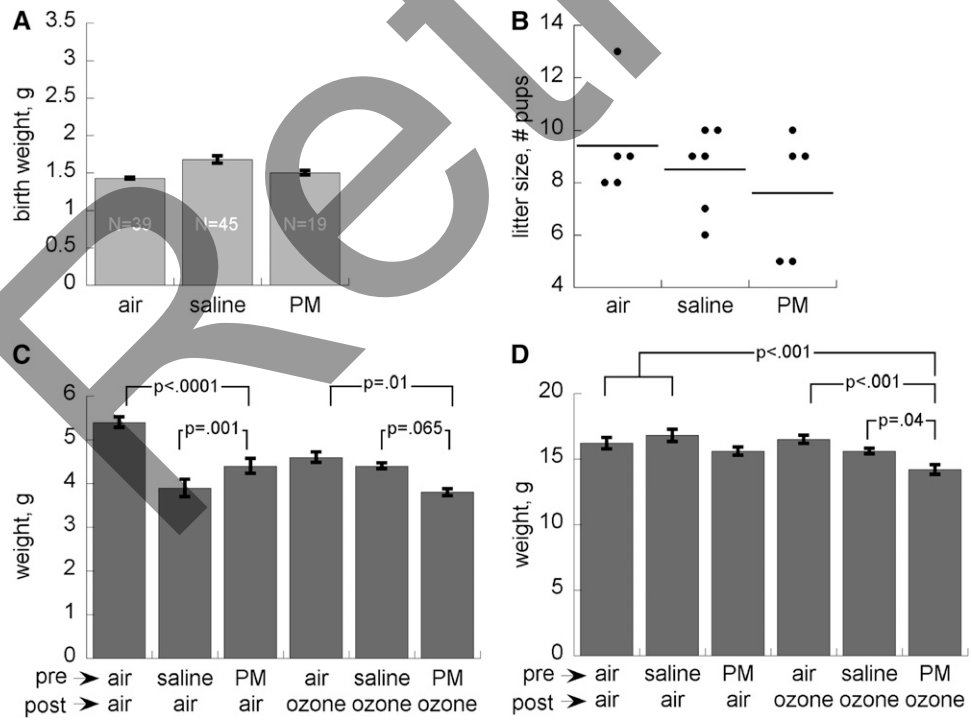

Figure 3. Effects of maternal treatment on (*A*) pup birth weight (mean ± SEM), (*B*) litter size (*bar* = mean), (*C*) postnatal body weights in air and ozone-treated pups at postnatal Day 8, and (*D*) postnatal Day 28. Data are means from 25 to 30 pups (from three litters) per treatment group ± SEM. PM = particulate matter.

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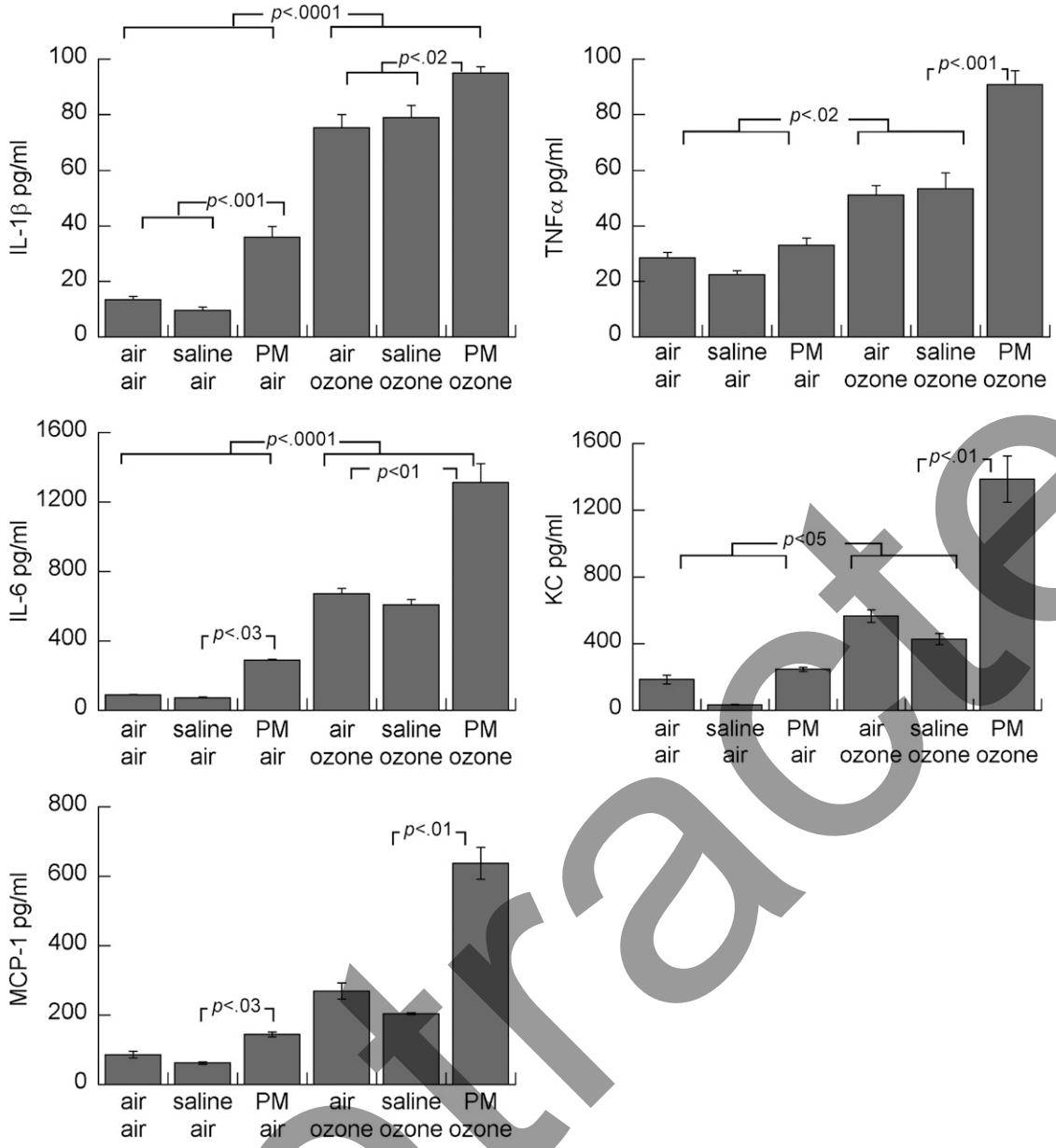

Figure 4. Effects of maternal treatments on whole lung cytokine concentrations in air- or ozone-exposed pups. Mean ± SEM; n = 4 to 5 per group. PM = particulate matter.

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increased in pups born to dams treated with PM during pregnancy. Maternal PM exposure also increased whole lung IL-1β and IL-6 in air-exposed pups. Leukocytes, predominantly mononuclear cells, were observed around some airways and in parenchyma, predominantly in ozone–exposed pups, but this was not quantified (*see* Figure E1 in the online supplement). Although neutrophils, identified by positive myeloperoxidase immunostaining, were more consistently observed in ozone–exposed pups (Figure E2), there were no obvious effects of prenatal treatment. Eosinophils were rarely observed in hematoxylin/eosin stained sections.

Effect of Postnatal Ozone Exposure with or without Maternal PM Exposure on Airway Hyperreactivity

Ozone increased AHR, but the increase was greatest in pups born to dams given PM. Prenatal and postnatal treatment had no effect on baseline total respiratory system resistance, R_{total}, or Newtonian (large airway) respiratory system resistance ($R_{Newtonian}$) (Figure 5). Changes in $R_{Newtonian}$ provoked by methacholine challenge were small compared with the changes in R_{total}. There were no effects of maternal treatment or postnatal ozone exposure on lung compliance.

Effect of Postnatal Ozone Exposure with or without Maternal PM Exposure on Airway Structure

Airways muscle bulk was assessed by anti–α-smooth muscle actin immunohistochemistry. Circumferential staining was rarely apparent in airways less than 100 μm in diameter (Figure E3). There were no obvious effects of treatment on airway smooth muscle thickness. Airway epithelial mucous metaplasia measured by the mean PAS score was highest in the group from PM-exposed dams exposed to postnatal ozone (Figure 6 and Figure E4). PAS staining was only evident in large (>100 μm) airways. There was only faint collagen deposition around small (<100 μm) airways, in contrast with larger airways and arteries (data not shown).

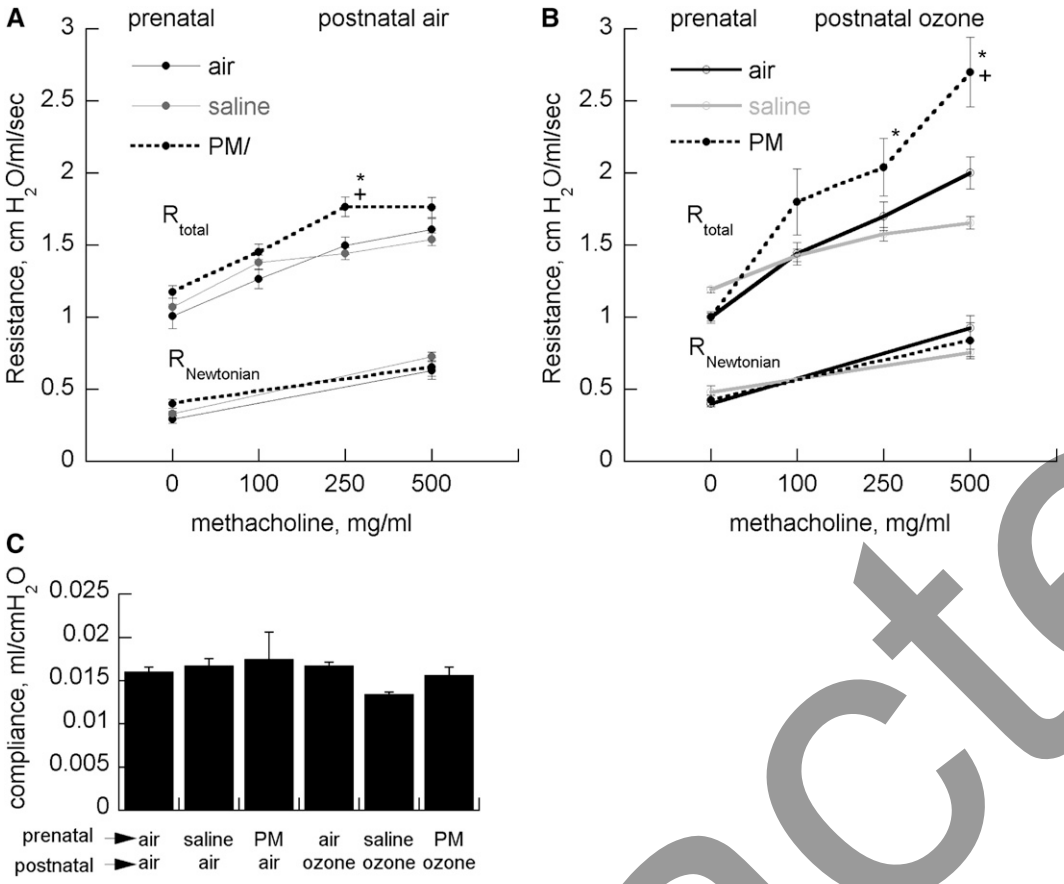

Figure 5. Effects of maternal treatment on total (R_{total}) or large airway ($R_{Newtonian}$) resistance in (*A*) air- or (*B*) ozone-treated pups treated with increasing concentrations of nebulized methacholine (mean, 20–25 per group) ± SEM. $^{+}P < 0.05$ vs. group born to air-treated dams; $^{*}P < 0.05$ vs. group born to saline-treated dams. (*C*) Effects of maternal treatment on respiratory system compliance (mean ± SEM). PM = particulate matter.

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Effect of Postnatal Ozone Exposure with or without Maternal PM Exposure on Alveolar Development

Because ozone can provoke inflammation (23), which could impair postnatal alveolar development (24), we measured alveolar volume density and surface density by stereologic morphometry. Ozone with or without prenatal PM exposure had no affect on alveolar volume or surface density (Figure 7). There were no qualitative effects on elastin deposition in alveolar septal tips (data not shown).

DISCUSSION

Maternal exposures are increasingly recognized as important modifiers of susceptibility to asthma, and ozone and PM have been associated with asthma exacerbations in children. We sought to determine whether the combination of maternal pulmonary exposure to a well-characterized urban particulate known to generate inflammation (25) and oxidative stress (26, 27) would augment postnatal ozone–induced AHR in offspring in C57BL/6 mice. We found that postnatal ozone exposure increased total airway resistance provoked by nebulized methacholine challenge compared with air-exposed control mice. AHR was exacerbated in juvenile mice born to dams treated with PM and exposed postnatally to ozone compared with those born to dams treated with saline and those born to dams treated with PM without postnatal ozone exposure.

The PM dose we used was relatively high, and it is possible that maternal insufflation with lower concentrations, or for fewer days during pregnancy, may not produce exacerbation of lung inflammation or ozone–induced AHR in offspring. PM exposure can be markedly increased by repeated exposures during severe pollution episodes (28) or under specific occupational conditions (29). It is important to determine if ambient inhalation of environmentally relevant particle concentrations produces the same effects.

We chose a well-characterized PM species, NIST SRM 1648 (described in detail in the online supplement), derived from urban PM for the maternal pulmonary exposure. Maternal ambient exposures to urban PM and other airborne pollutants have been associated with a number of adverse health effects in children, including low birth weight (30) and asthma (31), but the identities of the moieties that confer these health effects in humans has not been established (*see* Reference 32 for review). SRM 1648 lacks the organic compounds that would be found in higher abundance from PM obtained from modern traffic-related sources such as diesel exhaust particles (33), but both induce TNF-α, IL-6, and MIP-2 in leukocytes and pulmonary epithelial cells (34, 35). The maternal inflammatory response appears to be critical to the increased AHR observed in allergic sensitization models (*see* Reference 36 for review).

We used an ozone concentration previously used in newborn mice to elicit mild airway epithelial injury and inflammation (23, 37) and increased respiratory pause that has been associated with AHR (38). These concentrations are higher than human exposures because the deposition in rodents is lower than in humans (15). We used repetitive ozone exposures during the immediate postnatal period to model the repetitive nature of human exposures that can occur in early childhood. The effects of ozone exposure on lung development and AHR during this phase of lung development have not been described in detail, and it may be that repetitive exposures could desensitize the host to subsequent exposures. In adult humans, repetitive ozone exposures lead to diminution of the inflammatory responses and some pulmonary mechanics changes (39), but to our knowledge this has not been tested in juvenile rodents.

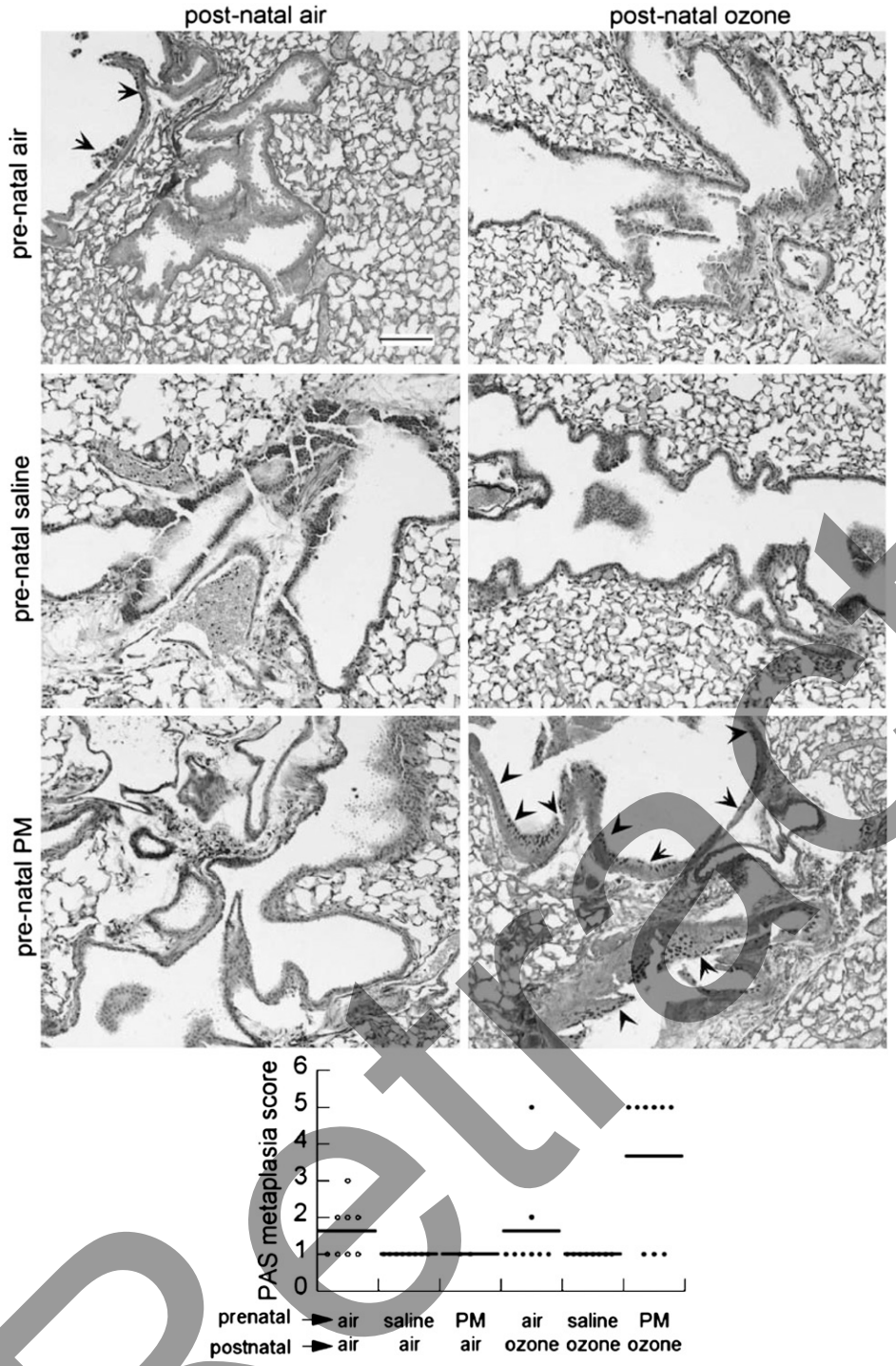

Figure 6. Effect of maternal treatments on periodic acid-Schiff (PAS) glycoprotein staining of airway epithelium in air-exposed or ozone-exposed pups. *Upper*: PAS epithelial cell staining (*arrowheads*) in airways of juvenile mice from PM- and saline-treated dams and exposed postnatally to ozone or air are shown in representative photomicrographs (*scale bar* = 100 μm). *Lower*: Histogram of PAS scoring of large airways scored in 6 to 8 pups per group (*line* = mean). PM = particulate matter.

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Mechanics measurements with forced oscillometry showed that the majority of the contribution of PM and ozone to methacholine-induced AHR could be attributed to small airways. The high-frequency measurements estimate so-called Newtonian or large airways resistance and were a relatively small contribution to total resistance. Alterations of lung tissue damping and tissue elastance were minimal (data not shown).

We observed that concentrations of 250 to 500 mg/ml of nebulized methacholine were required to elicit changes in total airway resistance in 28-day mice, which is higher than we have typically observed in adult C57BL/6 mice that demonstrate AHR in response to concentrations as low as 25 to 50 mg/ml. To our knowledge, there are no prior published studies of developmental effects on AHR responses to nebulized methacholine. We chose this age group and methacholine concentration range because our preliminary studies in 21-day mice showed minimal response to nebulized methacholine as high as 1,000 mg/ml (data not shown). In contrast, age had no effect on AHR sensitivity to intravenous methacholine in juvenile BALB/c mice that were assessed using forced oscillation, albeit without the neuromuscular blockade we used (40). It may be that there are genetic strain differences or that there are age-dependent effects on airway permeability to nebulized methacholine that account for these disparities. On the other hand,

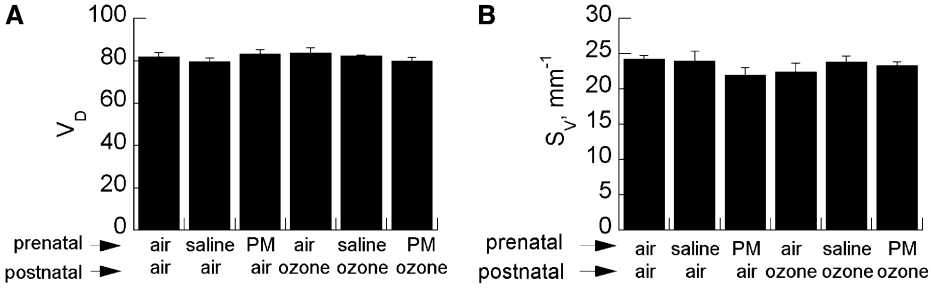

Figure 7. Effect of maternal treatment on (*A*) alveolar volume density (V_D) and (*B*) alveolar surface density (S_v) in pups exposed to air or ozone. Data are mean ± SEM (n = 5–6 per group group). PM = particulate matter.

susceptibility of juvenile mice to ozone-induced AHR has been suggested to depend on the age at exposure (38), but those observations relied on enhanced pause obtained by plethysmography, which is an indirect measurement of respiratory system mechanics. Our demonstration that postnatal ozone exposure enhances AHR using direct measurements of airway resistance is a novel finding.

Because the mechanics measurements showed that small airway resistance was the dominant contributor to AHR, we qualitatively assessed the anatomy of small airways, which are remodeled in adult mice after ovalbumin sensitization followed by chronic ozone exposure (41). There were no large, obvious changes in α-smooth muscle actin-labeled airway smooth muscle thickness. Even in large airways (>150 μm), smooth muscle was often discontinuous. This was found in all treatment groups. To our knowledge, ozone–induced airway smooth muscle changes have not been examined in juvenile mice. We did not perform a systematic, quantitative assessment, so we cannot exclude a contribution of smooth muscle bulk in small airways. Chronic ozone exposure in Rhesus monkeys alters the bulk and structure of small airway smooth muscle (42). We did not assess intrinsic muscle changes or alterations in signaling, which may have contributed to increased AHR, as has been shown in guinea pigs (43).

The role of alterations in airway smooth muscle structure/function in allergen-induced AHR in mice has been questioned (44). Other potential contributors include surfactant inhibition (45) and disrupted alveolar tethering due to dysanapsis (46). Ozone exposure has been shown to disrupt surfactant function, leading to respiratory alterations consistent with AHR in adult BALB/c mice (47), presumably through induction of inflammation. We found no effects of repeated ozone exposure (12 exposures over 4 weeks) on dynamic lung compliance in 28-day mice, so a contribution of surfactant disruption in this model system seems less likely. Likewise, we found no ozone–induced impairment of alveolar development, estimated by morphometry, which could theoretically have contributed to airway instability. We found no alterations in alveolar or subepithelial collagen deposition, as assessed by Masson trichrome staining. It is possible that there were more subtle anatomic changes to the airway structure, such as myofibroblast formation, that could have contributed to airway resistance. Although mucous cell metaplasia was increased in ozone–exposed pups born after maternal PM exposure, these changes occurred exclusively in large airways and likely would have contributed little to overall methacholine-provoked AHR.

We evaluated pulmonary inflammatory responses, some of which have been linked to ozone–induced AHR (48, 49). We found that there were increased numbers of leukocytes around small airways. We did not find increased eosinophils assessed in sections stained with hematoxylin and eosin. All of the proinflammatory cytokines we measured—IL-1β, TNF-α, KC, IL-6, and MCP-1—were elevated in whole lung homogenates from ozone–exposed juvenile mice, with more significant elevations in those born to PM-treated dams. In adult mice, AHR responses to chronic ozone exposure are dependent on Toll-like receptor 4 (TLR4) (11), the activation of which can signal increased expression of the panel of cytokines we tested (*see* Ref. 50 for review). We do not know if the TLR4 dependence of ozone–induced AHR is present in juvenile mice, nor is it understood by which mechanisms TLR4-activated signal transducers augment AHR.

Our finding that maternal pulmonary exposure to traffic-related pollutant particles augments AHR in offspring agrees with the findings of Hamada and colleagues (10) and Fedulov and colleagues (9), whose results, based upon measurement of enhanced expiratory pause, suggested that maternal inhalation of fly ash aerosol, or diesel exhaust particles, respectively, before birth augmented postnatal ovalbumin-sensitivity–induced respiratory reactivity. In those studies, pregnant mice demonstrated an exaggerated pulmonary inflammatory response to diesel particles and to putatively "inert" titanium dioxide particles compared with nonpregnant mice (9). We likewise observed a brisk influx of neutrophils in the bronchoalveolar lavage fluid of PM-exposed but not saline-treated pregnant mice. Our maternal PM instillations were conducted repetitively throughout pregnancy beginning in the first week. We speculate that this may have generated a sustained maternal pulmonary inflammatory response throughout pregnancy, but we did not evaluate this directly.

The mechanisms by which maternal pulmonary exposure to air pollutants might affect postnatal lung structure and function are poorly understood but likely include inflammation—not necessarily restricted to the lung (51)—as a common feature (52). In humans and mice, maternal-to-fetal transfer of inflammatory cytokines appears to be limited (53–55). Maternal-to-fetal transfer of susceptibility to allergen-induced AHR may depend on maternofetal transfer of immune cells (52) or IgG (56). We found that maternal PM instillation induced maternal pulmonary inflammation and fetal placental inflammatory cytokine expression. Our findings are consistent with those of Fujimoto and colleagues, who showed that maternal mouse inhalation of diesel particles at low concentrations induced placental expression of IL-2, -5, and -12, with high exposure inducing IL-6 expression in mice (57). The link, if any, between elevations of placental proinflammatory cytokines and the development of postnatal ozone–induced AHR is unclear, and the contributions of proinflammatory cytokines are complex. Genetic deletion or pharmacologic manipulation of several proinflammatory cytokine pathways (e.g., TNF-α receptor [58], IL-1β receptor [59], IL-17 [60]) decrease ozone–induced AHR.

Maternofetal transfer of PM-exposure–induced susceptibility could involve leaching of PM component(s) that traverse the placental circulation. It is not likely that the entire mass of the urban particle used in this study can enter the maternal pulmonary and systemic circulation in a significant quantity,

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but the metal components in the water soluble fraction may more easily permeate bronchiolar and alveolar capillary barriers via paracellular pathways or metal transporters. Fetal effects would depend on transplacental flux. Indirect effects could potentially induce oxidative stress and inflammatory cytokine production in the placenta.

Ozone alone may contribute to persistent susceptibility to AHR in young animals through effects on airway innervation. In infant Rhesus monkeys (61, 62), combined allergen-ozone exposure led to increased tracheal innervations, and ozone–exposed guinea pigs show increased sensitivity to efferent electrical stimulation of the airway (63). In adult guinea pigs, ozone–induced AHR is vagally mediated and partly dependent on eosinophil proximity to airway nerves (64). Maternal PM exposure–induced increases in airway leukocytes in close proximity to sensory nerves in offspring could contribute to increased sensitivity to ozone challenge (*see* Reference 65 for review).

In summary, we found that maternal pulmonary exposure to urban diesel PM exacerbated ozone–induced AHR to methacholine challenge in juvenile mice. Effects on AHR were predominantly in small airways, were accompanied by parallel increases in proinflammatory cytokines in whole lung homogenates, and increased peribronchiolar leukocyte accumulation. Changes in postnatal lung function were not accompanied by abnormalities in small airway structure. We speculate that pulmonary exposure to urban diesel PM during pregnancy may contribute to ozone–provoked asthma exacerbations in children, possibly through functional effects of inflammation on epithelial function. It is important to confirm these findings using other environmentally relevant particles and under ambient exposure conditions.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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