Ozone is a common urban air pollutant that remains a major health concern. Epidemiologic studies have demonstrated a strong association between high ambient O₃ concentration with cardiovascular and respiratory morbidity and mortality (Bell et al. 2005). O₃ exposure elicits airway inflammation characterized by neutrophil accumulation (Schelegle et al. 1991; Selzter et al. 1986) and liberates multiple inflammatory mediators, cytokines, and chemokines as an early inflammatory event (Aris et al. 1993; Koren et al. 1989). O₃-induced activation of airway neutrophilic infiltration is likely to produce additional damage through the release of reactive oxygen species and endogenous proteolytic enzymes.

Several different kinds of endogenous proteases participate in the pathogenesis of airway inflammation. Among these, matrix metalloproteinases (MMPs) belong to a family of zinc-dependent extracellular enzymes that share common structural features and have an important role in many physiological and pathological conditions (Parks and Shapiro 2001). MMPs are secreted by inflammatory and noninflammatory cells and have a major role in extracellular matrix (ECM) degradation, proteolytic modulation of biologically active proteins, and cell migration (Goetzl et al. 1996; Kelly et al. 2000; Kheradmand et al. 2002; Mautino et al. 1997; Parks and Shapiro 2001). Elevated levels of MMPs are prominent in inflammatory disorders of the airway and parenchymal lung disease and have been also implicated in several pulmonary diseases characterized by lung tissue damage, alveolar structure alterations, or abnormal repair (Elkington and Friedland 2006; Parks and Shapiro 2001). MMPs can be divided on the basis of their substrate specificity into collagenases (MMP-1, 8, 13), gelatinases (MMP-2, 9), stromelysins (MMP-3, 10, 11), matrilysin (MMP-7), macrophage metalloelastase (MMP-12), and membrane-type (MT)-MMPs (MMP-14, 15, 16, 17) (Visse and Nagase 2003).

Gelatinases degrade many ECM proteins; most important is type IV collagen, a major constituent of lung basement membrane (Nagase and Woessner 1999). Therefore, gelatinases have been thought to have an important role in the pathogenesis of various lung diseases (Nagase and Woessner 1999).

Gelatinases also can cleave a variety of non-ECM proteins, including cytokines and chemokines (McQuibban et al. 2000; Yu and Stamenkovic 2000; Zhang et al. 2003). MMP-2 (gelatinase A) is produced by a wide range of cell types in the lung, and it is increased in lung disorders with oxidative stress and inflammation etiologies (e.g., Parlo et al. 1998; Perez-Ramos et al. 1999; Tan et al. 2006). MMP-9 (gelatinase B) has been implicated in the pathogenesis of asthma (Caraldo et al. 2000), idiopathic pulmonary fibrosis (Fukuda et al. 1998), chronic obstructive pulmonary disease (COPD; Russell et al. 2002), and acute lung injury (Lanchou et al. 2003). In the normal lung, constitutive expression of MMP-9 is restricted to neutrophils and eosinophils. However, MMP-9 induced by inflammatory stimuli and proinflammatory cytokines is generated in multiple cell types including alveolar macrophages and other resident pulmonary cells (Atkinson and Senior 2003; Chakrabarti and Patel 2005). MMP-9 is known to be induced by O₃ in murine skin and lung (Kenyon et al. 2002; Valacchi et al. 2003).

MMP-7 has homeostatic function and a role in innate immunity in epithelial cells (Parks and Shapiro 2001). In adult human lung, MMP-7 is expressed by epithelial cells lining peribronchial and conducting airway (Dunsmore et al. 1998). Increased expression of MMP-7 has been observed in airway and alveolar cells at sites of lung cancer (Wilson and Matrisian 1996), idiopathic pulmonary fibrosis (Zuo et al. 2002), and cystic fibrosis (Dunsmore et al. 1998). Although it is clear that MMP-7 is associated with chronic lung
disease, the role of MMP-7 in O₃-induced airway inflammation is still unknown.

In the present studies, we hypothesized that murine MMP-7 and MMP-9 have important functional roles in facilitating the airway inflammation responses with O₃ relative to corresponding wild-type control mice.

### Materials and Methods

**Animals and inhalation exposure.** Male (6–8 weeks old, 20–25 g) C57BL/6J (Mmp7+/+) and B6.129-Mmp7<sup>tm1Lmm</sup>/J (Mmp7<sup>−/−</sup>), FVB/NJ (Mmp9<sup>+/−</sup>), and FVB.Cg-Mmp9<sup>−/−</sup>/J (Mmp9<sup>−/−</sup>) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Inhalation exposures were performed in a barrier facility at Alion Science and Technology, Inc. (Research Triangle Park, NC). After acclimation, mice were placed in individual stainless-steel wire cages within a Hazleton 1000 chamber (Lab Products, Maywood, NJ) equipped with a charcoal and high-efficiency particulate air-filtered supply. Mice had free access to water and pelleted open-formula rodent diet NIH-31 (Zeigler Bros., Inc., Gardeners, PA). Mice were exposed continuously to 0.3 ppm O₃ for 6, 24, or 48 hr, or 72 hr. O₃ was generated from ultra-high purity air (<1 ppm total hydrocarbons; National Welders, Inc., Raleigh, NC) using a silent air discharge O₃ generator (Model L-11; Pacific Ozone Technology, Benecia, CA). Constant chamber air temperature (72 ± 3°F) and relative humidity (50 ± 15%) were maintained.

O₃ concentration was monitored (Dasibi model 1008-PC, Dasabi Environmental Corp., Glendale, CA). Parallel exposure to filtered air was conducted in a separate chamber for 48 hr. All animal use was approved by the National Institute of Environmental Health Sciences Animal Care and Use Committee, and all animals were treated humanely and with regard for alleviation of suffering.

**Lung bronchoalveolar lavage fluid (BALF) and cell preparation.** Immediately after exposure to air or O₃, mice were euthanized with sodium pentobarbital (104 mg/kg). After 24- or 48-hr exposure, BAL was performed in situ 4 times with Hanks balanced salt solution (35 mM/kg; pH 7.2–7.4). These time points were chosen for BAL procedures on the basis of previous investigations with multiple inbred strains of mice that indicated inflammation and injury peaked between 24 and 48 hr during continuous exposure to 0.3 ppm O₃ (e.g., Cho et al. 2007; Dahl et al. 2007; Kleeberger et al. 1993). Recovered BALF was immediately cooled in ice and centrifuged (1,000 × g, 10 min). The cell pellets were resuspended in 1 mL of Hank’s balanced salt solution, and the cells were counted with a hemacytometer. Aliquots (150 µL) were cytocentrifuged, and the cells were stained with Wright-Giemsa stain solutions (Fisher Scientific, Pittsburgh, PA) for differential cell analysis (markers of cellular inflammation and epithelial injury). The supernatants from the first BALF return were assayed for total protein (a marker of lung permeability) with the Bradford assay (Bradford 1976).

**Lung tissue preparation for histopathology and immunohistochemistry.** Left lung lobes from mice exposed to either 0.3 ppm O₃ or filtered air for 48 hr were inflated gently with zinc formalin, fixed at a constant pressure (25 cm) of the same fixative for 30 min, tied off at the trachea, and immersed in the fixative for 1 day. Proximal and distal intrapulmonary axillary was excised, embedded in paraffin, and sectioned (5 µm). Tissue sections were histochemically stained with hematoxylin and eosin (H&E) for morphological comparison of pulmonary injury between genotypes. The terminal bronchioles and alveoli were the primary focus of study because 48-hr exposure to 0.3 ppm O₃ causes histologically evident inflammation and epithelial lesions in these regions of the mouse lung. Separate tissue sections from Mmp9<sup>+/+</sup> or Mmp9<sup>−/−</sup> mice were immunologically stained using a goat anti-mouse MMP-9 antibody (R&D Systems, Inc., Minneapolis, MN) or a rat anti-mouse Ki-67 antibody (Dako North American, Inc., Carpinteria, CA) to localize MMP-9 and Ki-67, respectively, using the standard peroxidase–diaminobenzidine (DAB) method.

**Western blot analysis.** Equal amounts of total lung protein (45 µg) isolated from mouse lung homogenates (air or 24, 48, and 72 hr of O₃) in radioimmunoprecipitation (RIPA) buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 10 g phenylmethylsulfonyl fluoride/ mL, 1 mM sodium orthovanadate, 1x protease inhibitor cocktail in 1x phosphate-buffered saline) were separated on 10–20% SDS gel electrophoresis gels. The 474–48–, and 72-hr time points were chosen for investigation to characterize the kinetics of lung MMP activity changes induced by O₃ exposure. Proteins were then transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked for 2 hr at room temperature with 5% nonfat milk in Tris-buffered saline–Tween buffer (20 mM/L Tris, 500 mM/L NaCl, 0.01% Tween 20). The blot was then incubated at 4°C overnight with anti-MMP-9 (R&D Systems), anti-MMP-2 (R&D Systems), or an anti-actin antibody (SantaCruz Biotechnology Inc., Santa Cruz, CA) as an internal control, followed by incubation for 1–2 hr with the proper secondary horseradish peroxidase-conjugated antibodies. The immunoblot was visualized through enhanced chemiluminescence.

**Total RNA isolation and real-time quantitative reverse transcriptase–polymerase chain reaction (RT-PCR).** Total RNA from frozen lungs (air or 6, 24, and 48 hr of O₃) was isolated using the RNeasy Midiprep kit (QIA-GEN, Valencia, CA). The 6-hr time point was included in this protocol to optimize the likelihood of capturing early inflammatory cytokine/chemokine gene expression on the basis of previous experience with this model (Cho et al. 2007). One microgram total RNA was reverse transcribed into cDNA in a volume of 50 µL containing 1x PCR buffer [50 mM KCl and 10 mM Tris (pH 8.3)], 5 mM MgCl₂, 1 mM each dNTPs, 125 ng oligo (dT)₁₅, and 50 µL of Moloney murine leukemia virus reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA), at 45°C for 15 min and 95°C for 5 min using the Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA).

An aliquot (2–5 µL) of the reverse transcriptase product (equivalent to 40–100 ng RNA) was amplified using PowerSYBR gene expression assays on the 7700 Prism sequence detection system (Applied Biosystems). The PCR conditions and data analysis were performed according to the manufacturer’s protocol in User Bulletin no. 2, “Applied Biosystems Prism 7700 Sequence Detection System” (Applied Biosystems 2007). Quantification of gene expression was determined by the number of cycles to threshold (Cₜ) of fluorescence detection. Relative gene expression was determined using the comparative Cₜ method as described previously (Chakrabarti and Patel 2005). Briefly, the ΔCₜ value was obtained by subtracting the 18s rRNA Cₜ value from the Cₜ value of the gene tested in the same sample. Message levels of each gene were expressed as fold changes relative to those in Mmp9<sup>+/+</sup> air controls.

**Gelatin zymography analysis.** Equal volumes of BALF (10 µL) samples were loaded on 10% Tris–glycine gel with 0.1% gelatin (Invitrogen Life Technologies) for electrophoresis. To eliminate the SDS content, the gels were washed with 2.7% (wt/vol) Triton X-100 at room temperature for 30 min and incubated for 16 hr in a buffer containing 50 mM Tris base, 40 mM HCl, 200 mM NaCl, 5 mM CaCl₂, and 0.02% (wt/vol) Brij 35 for gelatinolytic enzymes to act. The digested gels were stained in Colloidal Blue Staining kit (Bio-Rad Laboratories, Hercules, CA) for 4 hr to visualize the gelatin activity.
Gelatinolytic bands were analyzed by a Gel Doc 2000 System (Bio-Rad).

Cytokine enzyme-linked immunosorbent assay (ELISA). Immunoreactive macrophage inflammatory protein-2 [MIP-2, human interleukin-8 (IL-8) analogue, similar to chemokine (C-X-C motif) ligand (CXC) 1 and 2] and keratinocyte-derived chemokine [KC, human growth-regulated oncogene (GRO)-α analogue, similar to CXC1.1, 2, and 3] were quantified in aliquots of BALF (50 µL) using commercially available ELISA kits (R&D Systems), according to the manufacturer’s instructions. Each cytokine quantity was calculated from absorbance at 450 nm using a standard curve.

Statistics. All data are expressed as group means ± SE. Two-way analysis of variance (ANOVA) was used to evaluate the effects of subacute O₃ exposure on lung injury parameters between Mmp7+/+ and Mmp7−/− mice. The dependent variables were protein level, cell number, MMP-2 activity, and cytokine concentrations in BALF, and lung protein and mRNA levels. One-way ANOVA was used to evaluate the effects of O₃ on Mmp-9 production and expression in Mmp9+/+ mice. The Student–Newman–Keuls test was used for a posteriori comparisons of means. All analyses were performed using a commercial statistical analysis package (SigmaStat; SPSS Inc., Chicago, IL). Statistical significance was accepted at p < 0.05.

Results

Roles of Mmp-7 and Mmp-9 in O₃-induced airway inflammation. The roles of Mmp-7 and Mmp-9 in O₃-induced airway inflammation were assessed by exposing Mmp7+/+,

Mmp7−/−, Mmp9+/+, and Mmp9−/− mice continuously to air or O₃ for 24 and 48 hr. No significant differences in mean BAL protein concentration or cell numbers were found between respective +/+ and −/− genotypes after exposure to air (Figure 1, Table 1). After 48 hr of O₃ exposure, BAL protein concentration was significantly (p < 0.05) increased in all four genotypes (Figure 1, Table 1). The BALF protein concentration was significantly greater (68%) in Mmp7−/− mice compared with Mmp9−/− mice (Figure 1A); however, no significant differences in protein concentration were found between Mmp7+/+ and Mmp7−/− mice after exposure to O₃ (Table 1). O₃ caused significant increases in the mean numbers of BAL neutrophils in Mmp9+/+ and Mmp9−/− mice after 48 hr of O₃ exposure (p < 0.05; Figure 1B). However, compared with Mmp9−/− mice, O₃-enhanced BAL neutrophil numbers were significantly higher in Mmp9+/+ mice (p < 0.05). Mean numbers of epithelial cells were also significantly greater in Mmp7−/− mice relative to those in Mmp9−/− mice after 24- and 48-hr O₃ exposure (p < 0.05; Figure 1C). No significant differences in the number of BALF alveolar macrophages were found between Mmp9+/+ and Mmp9−/− mice (data not shown). No significant differences in mean numbers of BALF inflammatory cells were found between Mmp7+/+ and Mmp7−/− mice after exposure to O₃ (Table 1).

Effect of O₃ on MMP-2 and MMP-9 expression in the lung. We performed gelatin zymogram and Western blot analyses on BALF and lung protein extracts from Mmp9+/+ and Mmp9−/− mice to determine whether production of MMP-2 and MMP-9 was augmented by O₃ exposure. Both gelatinases have been implicated in the pathogenesis of inflammation-related lung diseases (e.g., Pardo et al. 1998; Tan et al. 2006); we also reasoned that MMP-2 expression might be enhanced to compensate for MMP-9 deletion. Minimal gelatinase activity was found in BALF from either genotype after air exposure. Exposure to O₃ (48 and 72 hr) caused significant (p < 0.05) increase in MMP-9 activity at 92 kDa (latent form) and 88 kDa (active form) in BALF from Mmp9+/+ mice (Figure 2A). Western blot analyses confirmed O₃-induced increase in MMP-9 protein levels in lung homogenates from Mmp9+/+ mice (Figure 2B). Furthermore, lung MMP-9 mRNA expression in Mmp9−/− mice was significantly increased compared with air controls after 6 hr of O₃ and remained increased by 48-hr exposure (p < 0.05; Figure 2C). MMP-9 proteins were localized primarily in alveolar epithelial cells, infiltrating inflammatory cells, endothelial cells, and cells in the injured parenchyma of Mmp9−/− mice after O₃ exposure (Figure 3).

Basal levels of MMP-2 protein and activity were detectable in lung protein extract and BALF, respectively, from Mmp9+/+ and Mmp9−/− mice, although no differences were detected between genotypes. O₃ increased MMP-2 activity and production in both genotypes compared with corresponding baseline (Figure 4A,B), but the increases were significantly (p < 0.05) greater in Mmp9−/− compared with Mmp9+/+ mice.

Effects of Mmp-9 deficiency on O₃-induced pulmonary pathology. H&E-stained lung sections from Mmp9+/+ and Mmp9−/− mice exposed to air were normal, and no histological differences were found between them (Figure 5A,5C). After 48 hr of O₃ exposure, significant peribronchiolar inflammation and proliferation as well as epithelial hyperplasia in terminal bronchioles were detected in Mmp9+/+ and Mmp9−/− mice (Figure 5B,D).

Table 1. Effect of Mmp7 deficiency on BALF protein and cell differentials after O₃ exposure (0.3 ppm for 48 hr).

| Genotype       | Proteins (µg/mL) | Macrophages (x10³/mL) | Neutrophils (x10³/mL) | Epithelial cells (x10³/mL) |
|----------------|------------------|-----------------------|-----------------------|-----------------------------|
| Mmp7+/+ (Air)  | 107.0 ± 6.7      | 21.8 ± 2.8            | 0.1 ± 0.1             | 1.7 ± 0.2                   |
| Mmp7−/− (Air)  | 140.0 ± 4.7      | 23.5 ± 1.9            | 0.02 ± 0.02           | 1.5 ± 0.3                   |
| Mmp7+/+ (O₃)  | 540.8 ± 32.1     | 47.8 ± 2.2*           | 5.8 ± 1.5*            | 3.0 ± 0.5                   |
| Mmp7−/− (O₃)  | 707.5 ± 73.1     | 49.7 ± 4.4*           | 10.0 ± 3.3*           | 7.1 ± 1.2*                  |

Results are the means ± SE for 5–8 mice in each group. *Significantly different from genotype-matched air control mice (p < 0.05).
However, O$_3$ caused more severe epithelial thickening, inflammation, and alveolar vacuolization in the terminal bronchioles of Mmp9$^{+/+}$ compared with Mmp9$^{−/−}$ mice (Figure 5D). Enhanced airway cellular proliferation was also more severe in the absence of MMP-9 as determined by immunohistochemistry of Ki-67–positive cells in G/S phases of the cell cycle (Figure 6). Nuclear localization of Ki-67 was mainly found in inflammatory cells and in cells of the terminal bronchioles that were injured by O$_3$.

**Effect of Mmp9 deficiency on protein levels and mRNA expression of the neutrophil chemokines KC and MIP-2.** To investigate the mechanism for increased neutrophil recruitment to the airway in the absence of MMP-9, we used ELISA to measure BALF levels of chemokines known to be involved in neutrophil chemotaxis (KC and MIP-2). No significant genotype-specific differences in mean concentrations of KC or MIP-2 were found after exposure to air (Figure 7). Mean KC and MIP-2 concentrations were significantly increased in BALF by O$_3$ exposure in both genotypes ($p < 0.05$, Figure 7). However, consistent with BALF inflammatory parameters and pathology (Figures 1, 5, and 6), KC and MIP-2 levels were significantly ($p < 0.05$) greater in Mmp9$^{−/−}$ mice compared with Mmp9$^{+/+}$ mice after 24- and 48-hr exposure to O$_3$ (Figure 7). KC and MIP-2 mRNA levels were not different between genotypes after exposure to air. After 24- and 48-hr exposure to O$_3$, KC and MIP-2 mRNA was increased significantly ($p < 0.05$) over the air controls in both genotypes but, interestingly, no differences were found between genotypes (Figure 8).

**Discussion**

O$_3$-induced airway inflammation is characterized by early neutrophil infiltration followed by a mononuclear cell dominated inflammation (Chitano et al. 1995). Molecular changes during the pulmonary pathogenesis induced by O$_3$ include increased production of prostaglandins, proinflammatory cytokines, and chemokines such as IL-6, IL-8, granulocyte macrophage colony-stimulating factor, KC, and MIP-2 (Devlin et al. 1991; Johnston et al. 2005a). In humans, neutrophil influx begins in peripheral airways as early as 6 hr after O$_3$ exposure and is known to be mediated by neutrophilic chemokines such as IL-8.
and GRO-α (Krishna et al. 1998). Functional roles of several inflammatory mediators such as tumor necrosis factor-α (TNF-α) (Cho et al. 2001; Kleeberger et al. 1997), IL-6 (Johnston et al. 2005b), and IL-1β (Arsalane et al. 1995), and the transcription factors nuclear factor kappa B (Cho et al. 2007; Laskin et al. 2002) and activator protein (AP)-1 (Cho et al. 2007) have also been determined in pulmonary inflammation of laboratory rodents exposed to O₃.

The primary functions of MMPs include degradation and turnover of ECM, tissue repair and remodeling, and leukocyte migration from peripheral circulation to inflammatory sites. Furthermore, recent findings suggested that MMPs can modulate inflammation and innate immunity by affecting the activity of various nonmatrix proteins (Parks et al. 2004). MMP-9 has been thought to be particularly important in the pathogenesis of inflammatory lung diseases, including acute lung injury, asthma, and COPD (Atkinson and Senior 2003). Higher than normal MMP-9 levels in the patients with these disorders may promote destruction of normal tissue architecture and increased migration of inflammatory cells to the disease sites (Lemjabbar et al. 1999a, 1999b).

In the present study, we hypothesized that MMP-9 is essential for O₃-induced airway inflammation because MMP-9 is one of the most predominant MMPs found in inflammatory airway diseases (Atkinson and Senior 2003). Contrary to predictions, we found that O₃-induced neutrophilic airway inflammation and injury were markedly increased in Mmp9⁻/⁻ mice compared with Mmp9⁺/⁺ mice, indicating a protective role of MMP-9 in...
O3-exposed airways. The results from the current study suggest that MMP-9 may not act primarily on ECM degradation that facilitates leukocyte migration to pulmonary inflammatory sites as indicated by another investigation (Betsuyaku et al. 1999). Heightened neutrophilic inflammation was also associated with enhanced levels of KC and MIP-2, which are important chemokines for neutrophil recruitment to the lung.

MMP-9 also processes CXC chemokines, which exert potent chemoattractant activities on leukocytes and alters their specific activities, but not CC chemokines (e.g., RANTES and MCP-2) (Van den Steen et al. 2000). MMP-9 truncates IL-8 (1–77) into IL-8 (7–77), which enhances neutrophil activation more than 10-fold (Van den Steen et al. 2000). In contrast, neutrophilic chemoattractant activity was decreased by MMP-9 degradation of the other CXC chemokines GRO-α and connective tissue–activating peptide (CTAP)-III (Van den Steen et al. 2000). Our current observation that O3 induced higher levels of BAL CXC chemokines KC and MIP-2 in Mmp9+/+ mice than in Mmp9−/− mice is consistent with, but does not prove, the notion that these chemokines were key effectors of pulmonary MMP-9. Interestingly, we also found no differences in the steady state mRNA levels of the O3-increased KC and MIP-2 between Mmp9−/− and Mmp9+/+ mice. Together these results suggest that differences in these chemokine levels in Mmp9−/− mice compared with those in Mmp9+/+ mice were caused by translational or posttranslational processes, which may include degradation/cleavage by MMP-9.

Significantly greater elevation of MMP-2 level and activity were observed in BAL CXC chemokines KC and MIP-2 between Mmp9−/− and Mmp9+/+ mice after O3 exposure. We cannot rule out the possibility that increased MMP-2 is a compensatory response in Mmp9−/− mice and may be involved in increased neutrophilic airway inflammation. However, MMP-9 has been reported to be the dominant airway MMP controlling inflammatory cell egression (Corry et al. 2004), and there is no evidence that MMP-2 has a modulating effect on inflammatory chemokines such as KC and MIP-2 (Parks et al. 2004). In addition, neutrophil concentrations in BALF were not changed in Mmp9−/− mice in a model of allergic asthma (Corry et al. 2002). We therefore postulate that MMP-2 does not have a critical role in heightened neutrophilic inflammation in Mmp9−/− mice, although it may be involved with other mechanisms of lung injury induced by O3 exposure (e.g., lung hyperpermeability).

The role of MMP-7 in acute lung injury has been studied in mouse models of interstitial pulmonary diseases such as fibrosis: Mmp7−/− mice had suppressed pulmonary fibrosis caused by bleomycin (Li et al. 2002; Zuo et al. 2002), and it was accompanied by decreased neutrophilic inflammation and chemokines (e.g., KC) in the alveolar fluid (Li et al. 2002). We predicted that O3-induced airway inflammation would be attenuated in Mmp7−/− mice. However, the current findings suggest that MMP-7 is not significantly associated with O3-induced airway inflammation and injury in mice. In addition, KC concentrations in BALF were not significantly different between Mmp7−/− and Mmp9−/− mice in the current model (data not shown). No studies have investigated the role of MMP-7 in oxidative lung injury such as O3. Different from MMP-9, matrikisin is produced by alveolar epithelial cells in injured lungs, and it has been thought to contribute to alveolar epithelial injury and re-epithelialization. Inhaled O3 mostly affects centriacinar regions of the airway, but not alveoli, which may partially explain why MMP-7 deficiency failed to alter airway inflammation by O3.

In summary, our results showed that deficiency in MMP-9 was associated with enhanced airway epithelial injury, neutrophil recruitment, and permeability following O3 exposure, but a deficiency in MMP-7 did not significantly affect O3-induced airway injury. The aberrant neutrophil recruitment was correlated with increased levels of KC and MIP-2 protein, but not mRNA expression, in the Mmp9−/− mice relative to Mmp9+/+ mice. Results are consistent with the hypothesis that enhanced O3-induced injury in Mmp9−/− mice is related to a difference in posttranscriptional processing of these CXC chemokines in the airway. These findings increase our understanding of the pathophysiological process of O3-induced lung injury and suggest that MMP-9 produced in the lung in response to oxidative stimuli may have a beneficial function.
Goetzl EJ, Banda MJ, Leppert D. 1996. Matrix metalloproteinases in immunity. J Immunol 156(1):1–4.

Johnston RA, Mirgzed JP, Shore SA. 2005. CCRX2 is essential for maximal neutrophil recruitment and methacholine responsiveness after ozone exposure. Am J Physiol Lung Cell Mol Physiol 288(1):L61–L67.

Johnston RA, Schwartzman IN, Fylen L, Shore SA. 2005b. Role of interleukin-6 in murine airway responses to ozone. Am J Physiol Lung Cell Mol Physiol 288(1):L61–L67.

Kelly EA, Busse WW, Jarrold NN. 2000. Increased matrix metalloproteinase-9 in the airway after allergen challenge. Am J Respir Crit Care Med 162(3 pt 1):1157–1161.

Kenyon NJ, van der Vliet A, Schok B, Okamoto T, McGrew GM, Last JA. 2002. Susceptibility to ozone-induced acute lung injury in INOS-deficient mice. Am J Physiol Lung Cell Mol Physiol 282(3):1540–1545.

Kheradmand F, Risik K, Werb Z. 2002. Signaling through the EGF receptor controls lung morphogenesis in part by regulating TGF-β-MMP-activated degradation of gelatinase A/MMP-2. Cell 115(4):689–699.

Kleberger SR, Leevic RG, Zhang Y-L. 1993. Susceptibility to ozone-induced inflammation. I. Genetic control of the response to subacute exposure. Am J Physiol 264(1 pt 1):15–20.

Kleberger SR, Leevic RG, Zhang Y-L, Lovegrove M, Harkema J, Jedlicka A. 1997. Linkage analysis of susceptibility to ozone-induced lung inflammation in inbred mice. Nat Genet 17(4):474–478.

Koren HS, Dewin RB, Graham DE, Mann R, McGee MP, Horstman DH, et al. 1989. Ozone-induced inflammation in the lower airways of humans. Am Rev Respir Dis 140(3):401–405.

Krishta MT, Madden J, Teran LM, Bisgonie GL, Lau LC, Withers NJ, et al. 1998. Effects of 0.2 ppm ozone on biomarkers of inflammation in bronchoalveolar lavage fluid and bronchial mucosa of healthy subjects. Eur Respir J 11(6):1294–1300.

Lanchon J, Corbel M, Tangy M, German N, Boichot E, Theret N, et al. 2003. Imbalance between matrix metalloproteinases (MMP-9 and MMP-2) and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 92(8):827–839.

Laskin DL, Fakhrazadeh L, Heck DE, Gerecke M, Laskin JD. 2002. Upregulation of phosphoinositide 3-kinase and protein kinase B in alveolar macrophages following ozone inhalation. Role of NF-kB and ERK-1/2 in ozone-induced nitric oxide production and toxicity. Mol Cell Biochem 234–235(1–2):251–258.

Lempiäär H, de Vos H, Lamblin C, Tillie J, Hartmann D, Waller A, et al. 1999. Contribution of 92 kDa gelatinase/type IV collagenase in bronchial inflammation during status asthmaticus. Am J Respir Crit Care Med 159(4 pt 1):1298–1307.

Levina M, Jaiswal A, Fattman CL, Niehouse LM, Tobolewski JM, Hanford LE, Li Q, et al. 2006. Matrix metalloproteinases promoted inflammation and fibrosis in asbestosis-induced lung injury in mice. Am J Physiol Lung Cell Mol Physiol 291(5):L739–L749.

Lipinski K, Warzocha D, Zawadzki J, Wozniak M, Markiewicz A. 2002. Ozone-induced airway inflammation in guinea pig is associated with increased expression of heme oxygenase-1. J Appl Physiol 92(1):39–47.

Lo Q, Park PW, Wilson CL, Parks WC. 2002. Matrikin shedding of syndecan-1 regulates chemokine mobilization and transcellular efflux of neutrophils in acute lung injury. Cell 111(5):835–846.

Mazzon G, Oliver N, Chancer P, Bousquet J, Capony F. 1997. Increased release of matrix metalloproteinase-9 in bronchoalveolar lavage fluid and by alveolar macrophages of asthmatics. Am J Respir Crit Care Med 155(5):853–581.

McQuibban GA, Gong JH, Tam EM, McCulloch CA, Clark-Lewis I, Overall CM. 2000. Inflammation dampened by gelatinase A cleavage of monoamine coactivator protein-3. Science 289(5482):1202–1206.

Mohan MJ, Seaton T, Mitchell J, Howe A, Blackburn K, Burkhart W, et al. 2002. The tumor necrosis factor-alpha converting enzyme (TACE): a unique metalloproteinase with highly defined substrate selectivity. Biochemistry 41(20):9642–9498.

Mohr R, Dhitala SK, Jung JC, Villar WV, McClure F, Russo LA, et al. 2002. Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epithelial regeneration. J Biol Chem 277(10):8055–8072.

Nogues H, Woeser P. 1999. Matrix metalloproteinases. J Biol Chem 274(31):21491–21494.

Pardo A, Barrios R, Maldonado V, Melendez J, Perez J, Ruiz IV, et al. 1998a. Gelatinases A and B are up-regulated in rat lungs by sub-acute hyperoxia: pathogenetic implications. Am J Pathol 153(3):833–444.

Parks WC, Shapiro SD. 2001. Matrix metalloproteinases in lung biology. Respir Res 2(1):10–19.

Parks WC, Wilson CL, Lopez-Baodo YS. 2004. Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat Rev Immunol 4(8):617–629.

Penner-Ramos J, Segura-Velarde MD, Vanda B, Selman M, Pardo A. 1999. Matrix metalloproteinases 2, 9, and 13, and tissue inhibitors of metalloproteinases 1 and 2 in experimental lung silicosis. Am J Respir Crit Care Med 160:1274–1282.

Peters JL, Sulsby CW, Friend J, Wormald P. 2002. Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol 26(6):620–629.

Piotrowicz R, Siwek AD, McDonald RJ. 1991. Time course of ozone-induced neutrophilic inflammation in normal humans. Am Rev Respir Dis 143(1):261–265.

Shonbeck U, Mach F, Libby P. 1998. Generation of biologically active IL-1 β by matrix metalloproteinases: a novel cascade-one-independent pathway of IL-1 β beta processing. J Biol Chem 273(9):5346–5349.

Seltzer J, Bigg BY, Stubing MB, Holtzman MJ, Nadel JA, Ueki IF, et al. 1996. O3-induced change in bronchial reactivity to methacholine and airway inflammation in humans. J Appl Physiol 80(4):1212–1225.

Sternlicht MD, Werz Z. 2001. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 17:460–516.

Tan RJ, Fatman CL, Niehouse LM, Tobolewski JM, Hanford LE, Li Q, et al. 2006. Matrix metalloproteinases promoted inflammation and fibrosis in asbestosis-induced lung injury in mice. Am J Respir Cell Mol Biol 35:289–297.

Valacchi G, Paggin E, Okamoto T, Corbacho AM, Diaro E, Davis PA, et al. 2003. Induction of stress proteins and MMP-9 by 0.8 ppm of ozone in murine skin. Biochem Biophys Res Commun 305(5):1741–746.

Van den Steen PE, Frosst P, Wuyts A, Van Damme J, Opdenakker G. 2002. Staphylococcal gelatinase B potentiates interleukin-8 tenfold by amnosomal processing, whereas it degrades CTAP-II, PF-4, and GRO-alpha and leaves RANTES and MCP-2 intact. Blood 96(6):2863–2871.

Veselkova J, Fazacek E, Frangos E, Hasselstrom P. 2003. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 92(8):827–839.

Wilson CL, Matrisian LM. 1996. Matrikines: an epithelial matrix metalloproteinase with potentially novel functions. J Invest Biol Cell Biol 28(2):123–136.

Yu Q, Stamenkovic I. 2000. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β-betas and promotes tumor invasion and angiogenesis. Genes Dev 14(2):163–176.

Zhang X, McQuibban GA, Silva C, Butler GS, Johnston JB, Holden J, et al. 2003. HIV-induced metalloproteinase processes of chemokines: a stromal cell derived factor-1 causes neurodegeneration. Nat Neurosci 6(10):804–1071.

Zuo F, Kaminski N, Eguil E, Allard J, Yakhnin Z, Ben-Dor A, et al. 2002. Gene expression analysis reveals matrikine as a key regulator of pulmonary fibrosis in mice and humans. Proc Natl Acad Sci USA 99(19):12692–12697.