CCL7 and CXCL10 Orchestrate Oxidative Stress-Induced Neutrophilic Lung Inflammation


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CCL7 and CXCL10 Orchestrate Oxidative Stress-Induced Neutrophilic Lung Inflammation^1


Oxidative stress from ozone (O3) exposure augments airway neutrophil recruitment and chemokine production. We and others have shown that severe and sudden asthma is associated with airway neutrophilia, and that O3 oxidative stress is likely to augment neutrophilic airway inflammation in severe asthma. However, very little is known about chemokines that orchestrate oxidative stress-induced neutrophilic airway inflammation in vivo. To identify these chemokines, three groups of BALB/c mice were exposed to sham air, 0.2 ppm O3, or 0.8 ppm O3 for 6 h. Compared with sham air, 0.8 ppm O3, but not 0.2 ppm O3, induced pronounced neutrophilic airway inflammation that peaked at 18 h postexposure. The 0.8 ppm O3 up-regulated lung mRNA of CXCL1,2,3 (mouse growth-related oncogene-α and macrophage-inflammatory protein-2), CXCL10 (IFN-γ-inducible protein-10), CCL3 (macrophage-inflammatory protein-1α), CCL7 (monocyte chemoattractant protein-3), and CCL11 (eotaxin) at 0 h postexposure, and expression of CXCL10, CCL3, and CCL7 mRNA was sustained 18 h postexposure. O3 increased lung protein levels of CXCL10, CCL7, and CCR3 (CCL7R). The airway epithelium was identified as a source of CCL7. The role of up-regulated chemokines was determined by administering control IgG or IgG Abs against six murine chemokines before O3 exposure. As expected, anti-mouse growth-related oncogene-α inhibited neutrophil recruitment. Surprisingly, Abs to CCL7 and CXCL10 also decreased neutrophil recruitment by 63 and 72%, respectively. These findings indicate that CCL7 and CXCL10, two chemokines not previously reported to orchestrate neutrophilic oxidative stress-induced neutrophilic airway inflammation. These observations may have relevance in induction of neutrophilia in severe asthma. The Journal of Immunology, 2002, 168: 846–852.

The worldwide incidence, prevalence, and mortality from asthma are increasing, but the cause of this phenomenon has not been elucidated. Previous reports suggest that the level of oxidative stress is increased in patients with asthma (1, 2). Markers of oxidative stress, such as 8-isoprostane and heme oxygenase-1, are increased in exhaled condensate and airway macrophages, respectively, of patients with asthma (1, 2). Exposure of patients to pro-oxidant environmental agents like O3 may contribute to worsening of oxidative stress in asthma and account for the increase in the incidence, prevalence, and mortality from asthma. This hypothesis is supported by epidemiological studies, which suggest that oxidative injury from O3 exposure can induce bronchospasm, decrease lung function, increase medication use, and increase hospitalization in patients with asthma (3–6).

Oxidative injury from O3 exposure has been shown to induce recruitment of neutrophils and decrement in lung function in patients with asthma (7, 8). Likewise, animal studies have demonstrated that O3 exposure potently induces recruitment and activation of neutrophils in the airways (9–12). Activation of neutrophils is likely to produce additional damage through the release of reactive oxygen species and proteolytic enzymes (7). We and others have shown that airway neutrophilia in asthma is associated with sudden onset fatality, severe asthma, and peribronchial fibrosis (13–18). These studies suggest that neutrophils may play an important role in severe asthma and support the concept that O3 oxidative stress-induced recruitment and activation of neutrophils may contribute to exacerbation of asthma.

Chemokines belong to a family of cytokines responsible for attracting inflammatory leukocytes, and some chemokines have been shown to be up-regulated in the lungs upon O3 exposure. Earlier studies have reported that O3 up-regulates CXCL-8 mRNA expression and protein production in the lungs of animals and humans exposed to O3 (19, 20). In addition to CXCL-8, O3 has been shown to induce other CXC chemokines, such as members of the Gro family of CXCL1,2,3 and CXCL10 (21–23). Other investigators have reported that O3 up-regulates CC chemokines such as CCL2, CCL3, and CCL11 (21, 23). Even though O3 exposure has been shown to up-regulate these chemokines, few studies have evaluated the in vivo role of these chemokines, which mediate O3-induced airway inflammation (12). The present study was designed to identify CC and CXC chemokines that have not been previously reported to mediate O3-induced airway inflammation. Here, we show that CCL7 and CXCL10, two chemokines not reported to mediate neutrophilic inflammation, orchestrate oxidative stress-induced neutrophilic airway inflammation.

Materials and Methods
Animal exposure to sham air or O3
Six- to 8-wk-old BALB/c mice (Harlan Laboratories, Indianapolis, IN) were placed in individual stainless steel wire-mesh cages with free access to food and water and were exposed to either sham air or O3 using Hinnert-type 0.85-m^3 stainless steel chambers. The inlet air was passed through...
activated charcoal and HEPA filters at sufficient flow to achieve chamber turnover rates of 30 volume changes/h. O₃ was generated from 100% O₂/He using a model 49 O₃ analyzer (Thermo Environmental Instruments, Franklin, MD). Mice were exposed for 6 h to either a low (0.2 ± 0.03 ppm) or high (0.8 ± 0.03 ppm) dose of O₃. After exposure, sham and O₃-exposed animals were sacrificed, and bronchoalveolar lavage (BAL)³ was performed at 0, 18, 42, and 138 h after exposure cessation (Fig. 1).

**BAL and cell count**

Cellular infiltration into the airway lumen was assessed by an analysis of BAL fluid. Mice were anesthetized with an i.p. injection of ketamine and xylazine. A tracheostomy was performed and the trachea cannulated. BAL of the lung was performed twice with 0.7 ml of sterile PBS (pH 7.3) through the trachea cannula with a syringe. The BAL was centrifuged at 4°C for 10 min at 400 g, and the pellet was suspended in 750 µl of ice-cold Dulbecco’s PBS (Sigma-Aldrich, St. Louis, MO). Total cell counts in BAL were determined from an aliquot of the cell suspension. Differential cell counts were done on cytocentrifuge preparations (Cytospin 3; Thermo Shandon, Pittsburgh, PA) stained with Wright-Giemsa, counting 200 cells from each animal.

**Analyses of lung chemokine mRNA levels by RT-PCR**

Chemokine mRNAs were assessed by semiquantitative RT-PCR. Immediately after the mice were sacrificed, the lungs were removed, frozen rapidly in liquid nitrogen, and stored at -80°C until RNA extraction. The frozen whole lung was placed into 2 ml of TRIzol solution (Life Technologies, Carlsbad, CA) and total RNA was isolated following the manufacturer’s protocol. Five micrograms of total RNA was reverse transcribed using 200 U of SuperScript II Reverse Transcriptase in a buffer (Life Technologies) containing 10 mM DTT, 1 µM oligo(dT) primer (Life Technologies), 500 µM of each dNTP, and 10 U of RNasin (Promega, Madison, WI) in a total volume of 20 µl. The resultant cDNA was diluted 5-fold with diethyl pyrocarbonate-treated water to a final volume of 100 µl. To amplify the reverse-transcribed cDNA, 4 µl of 1:1 PCR mixture was subjected to a final solution containing 1× Taq buffer II, 2.0 mM MgCl₂, 0.2 µM of each of sense and antisense primer, and 0.5 U of AmpliTaq Gold polymerase (PerkinElmer/Applied Biosystems, Foster City, CA). PCR was performed in GeneAmp PCR system 9700 (PerkinElmer/Applied Biosystems). All primers were custom-designed using the Primer software program (Table I). The number of amplification cycles used ranged from 23 to 26. The number of amplification cycles was selected for each primer based on the linear portion of the PCR amplification cycle. The PCR products were electrophoresed in a 3% agarose gel (Seakem LE Agarose; FMC Bioproducts, Rockland, ME). The intensity of bands on photographs of the agarose gel was quantified by scanning the photographs with a contrast scanner (JX-330; Sharp Electronics, Mahwah, NJ) using optical software (ImageQuant, version 3.3; Molecular Dynamics, Sunnyvale, CA). The values obtained from individual cytokines were expressed as a ratio of cytokine band intensity relative to band intensity of housekeeping gene, β₂-microglobulin (β₂m).

**Lung CXCL10 (IFN-γ-inducible protein (IP)-10), CCL7 (monocyte chemotactic protein (MCP)-3), and CCR3 protein levels**

Whole lung lysates of mice exposed to sham air or 0.8 ppm O₃ for 6 h were subjected to denaturing gel electrophoresis. To detect these chemokines or chemokine receptors, Western blotting was performed using rabbit anti-murine chemokine Abs (PeproTech, Rocky Hill, NJ) or rabbit anti-murine chemokine receptors, Western blotting was performed using rabbit anti-murine chemokine Abs (PeproTech) or murine chemokine Abs (PeproTech). The tissue-bound anti-CCL7 was detected with Alexa 488-conjugated, highly cross-absorbed goat anti-rabbit IgG (Molecular Probes, Eugene, OR). The sections were visualized in an inverted TE200 Nikon Fluorescent microscope attached to a Photometrics Cool Snap Fx CCD digital camera and Metamorph software (Roper Scientific, Tucson, AZ).

**Administration of anti-chemokine Abs**

Rabbit IgG (control) and rabbit IgG Abs against CXCL1,2,3 (mouse growth-related oncogene-α (KC), macrophage-inflammatory protein (MIP)-2), CXCL10 (IP-10), CCL3 (MIP-1α), CCL7 (MCP-3), and CCL11 (eotaxin) (Peprotech) were administered to naive BALB/c. All Abs were administered intranasally (i.n.) at a concentration of 10 µg/100 µl PBS 1 h before O₃ exposure (Fig. 1B). We and others have previously shown that anti-chemokine Abs are very effective in preventing lung inflammation in vivo when they are administered directly into the lungs (24, 25). For this reason, we administered Abs (rabbit polyclonal IgG) directly into the lungs in this study. The mice were exposed to 0.8 ppm O₃ for 6 h and were sacrificed to perform BAL at the anticipated peak of lung inflammation, that is 18 h after the end of O₃ exposure.

**Speciﬁcity of rabbit anti-murine chemokine Abs**

To determine speciﬁcity of rabbit anti-mouse chemokine Abs, we performed denaturing gel electrophoresis of recombinant murine CXCL10 (IP-10), KC, CCL7 (MCP-3), and CCL5 (RANTES), followed by Western blotting using rabbit Abs directed against murine CXCL10, KC, and CCL7 (Peprotech). Data analysis

Data are presented as mean ± SEM. The difference in outcome variables between treatment groups was analyzed by one-way ANOVA. Significant ANOVAs were further analyzed by the Bonferroni/Dunn’s post hoc test.

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³ Abbreviations used in this paper: BAL, bronchoalveolar lavage; i.n., intranasally; β₂m, β₂-microglobulin; IP, IFN-γ-inducible protein; MCP, monocyte chemotactic protein; KC, mouse growth-related oncogene-α; MIP, macrophage-inflammatory protein.
Results

High-dose (0.8 ppm) but not low-dose (0.2 ppm) O₃ exposure induces neutrophilic and macrophagic airway inflammation

Sham air exposure of BALB/c mice did not alter the number of neutrophils, eosinophils, macrophages, or lymphocytes in the recovered BAL at any time. Exposure of mice to a low concentration (0.2 ppm) of O₃ failed to induce a detectable increase in any of these cell populations within the lungs. In contrast, exposure of mice to a high concentration of O₃ (0.8 ppm) increased the total cells maximally by 18 h (air = 2.57 ± 0.53 × 10⁴/ml vs 0.8 ppm O₃ = 23.43 ± 4.99 × 10⁴/ml; p ≤ 0.01; Fig. 2). The same high concentration of O₃ increased the number of neutrophils at 18 h (air = 2.52 ± 0.52 × 10⁴/ml vs 0.8 ppm O₃ = 13.77 ± 3.37 × 10⁴/ml; p ≤ 0.01; Fig. 2). These data indicate that 0.8 ppm O₃ for 6 h increased total cells, neutrophils, and macrophages as early as 18 h postexposure, and both cell types demonstrated sustained increase until 42 h postexposure.

High dose of O₃ up-regulates expression of CXCL10, CCL7, and other chemokines

RT-PCR of lung mRNA was performed to measure expression of CXC and CC chemokines, and the data of two representative chemokines, namely, CXCL10 and CCL7, are shown in Fig. 3. The ratio of intensity of chemokine band to the intensity of the housekeeping gene β₂m was determined (Fig. 4). Exposure of mice to 0.8 ppm O₃ for 6 h up-regulated some chemokine genes at 0 h postexposure, which did not persist at 18 h postexposure. These include the CXCL1,2,3 (KC, 10.5-fold increase), MIP-2 (18.3-fold increase), and CCL11 (9.1-fold increase). Exposure of mice to the same dose of O₃ for 6 h up-regulated other genes at 0 h postexposure, but these genes remained up-regulated at 18 h postexposure. This group of chemokines includes CXCL10 (10.6-fold increase), CCL3 (3.9-fold increase, p ≤ 0.001), and CCL7 (13.1-fold increase, p ≤ 0.0001). The expression of the second group of chemokine genes (CXCL10, CCL3, and CCL7) was up-regulated 3.3-, 2.1-, and 4.5-fold, respectively, at 18 h. These results indicate that some genes remained highly expressed at 18 h postexposure.

Table 1. Primers for PCR

<table>
<thead>
<tr>
<th>Chemokines</th>
<th>Forward Primers</th>
<th>Reverse Primers</th>
</tr>
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<tbody>
<tr>
<td>CXCL1,2,3 (KC)</td>
<td>CCA ACA CAG CAC CAT GAT CC</td>
<td>CCT CCG GAC CAT TCT TG</td>
</tr>
<tr>
<td>CXCL1,2,3 (MIP-2)</td>
<td>ACA CAT CCT AGG GCC AT</td>
<td>GGT CAG TTA GCC TTG CCT</td>
</tr>
<tr>
<td>CXCL10 (IP-10)</td>
<td>CCA TCA GCA CCA TGA ACC</td>
<td>TCC GGA TCC AGA CAT CTC</td>
</tr>
<tr>
<td>CCL3 (MIP-1α)</td>
<td>CAC TCT GCA ACC AAG TCT TC</td>
<td>TCA GTT CCA GGT CAG TGA TG</td>
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<td>CCL7 (MCP-3)</td>
<td>AGC TAC AGA AGG ATC ACC AG</td>
<td>CAC ATT CCT ACA GAC AGC TC</td>
</tr>
<tr>
<td>CCL11 (eotaxin)</td>
<td>GCT CCA CAG GCC TTC TTA GCC TTG CCT</td>
<td>TGG TGG CAT CCT GGA CC</td>
</tr>
<tr>
<td>β₂m</td>
<td>ATG GCT CGC TCG GTG ACC ACC CTA G</td>
<td>TCA TGA TGC TTG ATC ACA TGT CTC G</td>
</tr>
</tbody>
</table>

FIGURE 2. BAL cell counts in BALB/c mice exposed to O₃ or sham air. BALB/c mice were exposed to O₃ (0.2 or 0.8 ppm) or sham air for 6 h. After exposure, sham- and O₃-exposed animals were sacrificed, and BAL was performed at 0, 18, 42, and 138 h after the cessation of O₃ exposure. The numbers of cells were calculated as the product of the total cell count and the differential count of cytospin slides stained with Wright-Giemsa stain. Values shown represent mean ± SEM. Asterisks indicate significant differences (*, p ≤ 0.05; **, p ≤ 0.01) and represent difference in cell influx associated with clean air vs O₃ exposure.

FIGURE 3. RT-PCR of total lung mRNA. BALB/c mice were exposed to 0.8 ppm O₃ or sham air for 6 h. The animals were sacrificed 0 h or 12 h later. The numbers 1–12 represent individual animals. Animals 1–3 were exposed to air and sacrificed at 0 h postexposure. Animals 4–6 were exposed to 0.08 ppm O₃ for 6 h and sacrificed at 0 h postexposure. Animals 7–9 were exposed to air and sacrificed at 18 h postexposure. Animals 10–12 were exposed to 0.08 ppm O₃ and sacrificed at 18 h postexposure. Total RNA was extracted from the lung of all animals. RT-PCR was performed to amplify the chemokine and β₂m RNA (see Table I for primer sequence). This figure shows representative data of two chemokines, namely, CXCL10 and CCL7. The presence of β₂m bands in all animals indicates that mRNA extraction and cDNA synthesis was performed successfully.
CXCL1,2,3 and CCL11) are only transiently up-regulated by O₃ exposure, whereas others (CXCL10, CCL3, and CCL7) are up-regulated for a prolonged period (Figs. 3 and 4).

**High dose of O₃ increases CXCL10, CCL7, and CCR3 protein levels in the lungs**

Because exposure of mice to sham air or 0.8 ppm O₃ for 6 h up-regulated CXCL10 and CCL7 mRNA levels, we sought to determine whether it also increased protein levels of these chemokines. As shown in Fig. 5A, 0.8 ppm O₃ dramatically increased lung levels of both CXCL10 and CCL7. Furthermore, 0.8 ppm O₃ also increased lung levels of CCR3, the receptor for CCL7 (Fig. 5B).

**O₃ increases CCL7 production from airway epithelium**

We sought to determine the cell source of CCL7. As shown in Fig. 6, exposure of mice to air failed to induce production of CCL7. Exposure of mice to 0.8 ppm O₃ increased epithelial levels of CCL7 (Fig. 6).

**CXCL10 and CCL7 mediate O₃-induced neutrophilic inflammation**

To evaluate the role of the six chemokines up-regulated by O₃ in mediating airway inflammation induced by O₃, seven groups of mice were treated i.n. with normal rabbit IgG (isotype control) or polyclonal rabbit anti-mouse IgG against one of six murine chemokines, namely, CXCL1,2,3 (KC and MIP-2), CXCL10, CCL3, CCL7, and CCL11 (Fig. 7). Exposure of control IgG-treated mice to 0.8 ppm O₃ for 6 h induced a vigorous inflammatory response by 18 h postexposure. As expected, the Ab to KC decreased the number of total cells by 64% (*p < 0.01) and neutrophils by 73% (*p < 0.01). Surprisingly, the Ab to CXCL10 also decreased total cells by 58% (p < 0.05) and neutrophils by 72% (p < 0.01), whereas the Ab to CCL7 decreased neutrophils by 63% (p < 0.05). Abs to MIP-2, CCL3, and CCL11 did not inhibit recruitment of either neutrophils or total cells. Macrophagic inflammation was not significantly attenuated by any of these Abs (data not shown).

**Specificity of anti-murine chemokine Abs**

Because cross-reactivity of anti-murine CXCL10 and anti-CCL7 with KC could explain our unexpected observations of the effects of these Abs on neutrophil recruitment, we sought to examine the specificity of anti-murine chemokine Abs

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**FIGURE 4.** Intensity of cytokine PCR bands. The intensity of cytokine and β₂m PCR bands in the original photographs of the agarose gel was quantified by scanning the photographs with a contrast scanner and analyzing intensity by ImageQuant optical software. The ratios of CXCL1,2,3 (KC) (A), CXCL1,2,3 (MIP-2) (B), CXCL10 (C), CCL3 (D), CCL7 (E), and CCL11 (F) band intensity to β₂m band intensity are shown. Values are expressed as the mean ± SEM. Asterisks indicate significant differences (*, p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.001).
specificity of these Abs. We performed denaturing gel electrophoresis of recombinant chemokines followed by Western blotting using rabbit anti-murine chemokine Abs. As shown in Fig. 8, anti CXCL10 recognized CXCL10 but not KC, CCL7, and CCL5. Likewise, the Abs to CCL7 and KC specifically recognized CCL7 and KC, respectively.

Discussion
A notable novel finding in our study is that oxidative stress from O₃ exposure up-regulated CCL7 mRNA expression and protein production in the lungs. The airway epithelium was a cell source of CCL7 in the lungs. Exposure of humans to low concentrations of O₃ has recently been shown to increase allergen-induced eosinophil inflammation, an observation that may explain why O₃ exposure worsens asthma (26, 27). We have previously reported that allergen challenge induces CCL7 production from the airway epithelium and that CCL7 mediates eosinophil inflammation in a mouse model of asthma (25). The O₃ exposure-induced production of CCL7 may explain how O₃ exposure increases allergen-induced eosinophil inflammation in allergic subjects and worsens asthma (26). Another unexpected novel finding in our study is that the Ab to CCL7 inhibited O₃-induced neutrophilic lung inflammation. This observation is quite surprising because CCL7 has been reported to bind to CCR3, a CCR that is reportedly present on Th2 cells and eosinophils, but not on neutrophils (28–30). This paradox may be partially explained by a recent report that even though neutrophils did not express CCR3 under basal condition, they expressed high levels of this receptor when they are activated (28). Although we did not examine CCR3 expression on lung neutrophils, our data indicate that CCR3 protein level is dramatically increased in the lungs 18 h after O₃ exposure, at a time point when the lungs are heavily infiltrated with neutrophils. Alternatively,
CCL7 may facilitate neutrophil chemotraction by a second chemokine, similar to the facilitatory role of CCL7 in neutrophil chemotraction induced by the chemokine RAGAKINE-1 (31).

Few investigators have evaluated the in vivo role of chemokines in mediating O3-induced airway inflammation. Koto et al. (12) reported that in vivo treatment with an Ab to the Gro family (anti-cytokine-induced neutrophil chemotactant analogous to MIP-2) inhibited neutrophilic inflammation but not other cell types in rats. Consistent with the observations of Koto et al., we found that an Ab to the Gro family (KC) inhibited neutrophil recruitment. Unexpectedly, we also found that Abs to CCL7 and CXCL10 significantly inhibited O3-induced neutrophilic inflammation. The role of CXCL10 in neutrophil recruitment has not been described previously, but CXCL10 can be produced by neutrophils, and therefore can influence chemotaxis of CXCR3-expressing cells such as Th1 cells, which in turn may influence and regulate neutrophilic inflammation (32).

In the present study Abs directed against multiple chemokines, namely, CXCL10, CCL7, and KC, significantly inhibited ozone-induced neutrophil accumulation in the lung. An understanding of the specific roles of chemokines in orchestrating airway inflammation has been complicated by a number of different factors (33). First, simultaneous expression of multiple chemokines with partially overlapping functions is often observed. Second, chemokines often bind more than one chemokine receptor, and chemokine receptors typically bind more than one class of chemokine. Third, a likely scenario is that coordinated chemokine actions may have contributed to the reduced neutrophilic lung inflammation, as suggested by a study examining the roles of chemokines in allergic asthma. In that study, neutralization of several chemokines including CCL2, CCL3, CCL11, CCL12, and CCL22 was effective in inhibiting cellular recruitment (34). In this study, the chemokines were found to act in a coordinated fashion in contributing to the complex pathophysiology of allergic inflammation. In the current study, anti-chemokine Abs were administered 1 h before ozone exposure, and lung neutrophil accumulation was assessed 18 h after ozone exposure. During this interval, different chemokines may have influenced distinct processes underlying ozone-induced neutrophilic lung accumulation. In a framework of a coordinated chemokine network, the protocol of administration of the Abs in this study does not allow assessment of variable chemokine temporal activity or spatial activity because the Abs were administered before ozone exposure and ultimately to many sites in the lung via the i.n. route.

Oxidative stress from O3 exposure has been reported to induce airway hyperresponsiveness in humans (36, 37). These authors have suggested that because of these effects, O3 exposure is likely to contribute to asthma morbidity. Oxidative injury from O3 exposure has also been shown to induce recruitment of neutrophils in humans (7). Because severe and sudden-onset asthma is associated with airway neutrophilia, O3 exposure is likely to contribute significantly to asthma morbidity and mortality in this subset of patients (13–18). Based on the data presented in this study, it is tempting to speculate that CXCL10 and CCL7 contribute to airway neutrophilic inflammation and asthma exacerbation in these patients after exposure to high O3-polluted air.

The Office of Air Quality Planning and Standards has set the National Ambient Air Quality Standards “criteria pollutants,” including O3. The 1-h standard is 0.12 ppm, whereas the 8-h standard is 0.08 ppm. These levels are considerably lower than the dose that induced airway inflammation in the present study (0.8 ppm) and in previous studies involving exposure of mice to O3 (9, 35). These differences may represent a greater response of humans to O3 than mice, rats, and guinea pigs, as suggested by the observation that humans demonstrate an increase in neutrophilic inflammation even when they are exposed to 0.2 ppm O3 (8). These findings suggest that caution should be exercised when extrapolating murine data to humans. Nonetheless, the results of the present study suggest that future studies in humans should be directed at evaluating the role of CCL7 and CXCL10 in O3 oxidative-stress-induced lung inflammation.

In summary, our results indicate that high concentrations of O3 increase recruitment of neutrophils and macrophages into the lungs, associated with an increase in expression of CXCL1,2,3 (KC and MIP-2), CXCL10, CCL3, CCL7, and CCL11. Exposure to 0.8 ppm O3 also increased lung protein levels of CXCL10, CCL7, and CCR3. In addition to the expected inhibition of neutrophil recruitment by the Ab to KC, administration of neutralizing Abs to murine CCL7 and CXCL10 before O3 exposure inhibited O3-induced neutrophil recruitment. These findings indicate that CCL7 and CXCL10 orchestrate oxidative stress-induced neutrophilic lung inflammation. This is the first report demonstrating an in vivo role of these chemokines in neutrophilic inflammation.
These observations may have relevance to induction of neutrophilia in severe asthma.

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References