Complement Activation Is Critical to Airway Hyperresponsiveness after Acute Ozone Exposure

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Ozone (O_3) can induce airway hyperresponsiveness (AHR) and neutrophilic inflammation. We evaluated the role of complement in development of AHR and inflammation after acute O_3 exposure in mice. Mice were exposed to O_3 at 2 ppm for 3 hours, and airway responsiveness to methacholine was measured 8 hours after O_3 exposure. Complement was depleted or inhibited by intraperitoneal injection of cobra venom factor (CVF) or complement receptor–related gene y (Crry)–Ig, a potent C3 convertase inhibitor; neutrophils were depleted using an antineutrophil monoclonal antibody. CVF attenuated the development of AHR by O_3. Administration of Crry–Ig also prevented the development of AHR. Bronchoalveolar lavage (BAL) fluid neutrophilia after O_3 exposure was significantly decreased by administration of either CVF or Crry–Ig. Increased BAL fluid total protein after O_3 exposure was lowered by depletion or inhibition of complement. In contrast to the effects of complement inhibition or depletion, depletion of BAL neutrophil counts by more than 90% with the monoclonal antibody did not affect the development of AHR after O_3 exposure. These data indicated that activation of the complement system follows acute O_3 exposure and is important to the development of AHR and airway neutrophilia. However, this neutrophil response does not appear necessary for the development of AHR.

Keywords: ozone; complement activation; airway hyperresponsiveness

Ozone (O_3) is a toxic oxidant found increasingly in urban environments and workplaces (1). Cumulative data from a number of laboratories implicate O_3 in airway inflammation and airway hyperresponsiveness (AHR) (2–7). O_3 exposure rapidly causes damage to epithelial cells and alveoli, and this has been associated with development of AHR. The acute response to O_3 and likely to the acute injury is the generation and release of a number of cytokines and chemokines, proinflammatory mediators, and a significant influx of neutrophils (2, 3, 5–10).

A number of earlier reports implicated this O_3-induced accumulation of neutrophils into the airways as critical to the development of AHR (2, 11). In more recent studies, this association between neutrophils and AHR has not been substantiated (3, 4, 8, 12, 13). In their place, other factors have been linked to susceptibility to O_3-induced AHR. Prominent among these is tumor necrosis factor receptor-2 (5, 6), with susceptibility linked to tumor necrosis factor-α (TNF-α) receptor expression and signaling (14).

In a number of different models of AHR, including allergen-induced or immune complex–triggered AHR, increasing attention has been focused on the complement system and complement activation products that are involved in neutrophil chemoattraction (15–21). Airway epithelial cells may be a source of these factors (22, 23), for example, when injured after O_3 exposure. In humans exposed to O_3 (0.4 ppm for 2 hours), increased levels of C3a were detected (24). In this study, we examined the effects of complement depletion with cobra venom factor (CVF) or an inhibitor of complement activation (complement receptor–related gene y [Crry]–Ig) in the neutrophil response to acute O_3 exposure as well as in the development of AHR.

METHODS

Animals

Eight- to 12-week-old female C57BL/6j mice, mast cell–deficient mice ([WB/Rej-kit^{W}/+ × C57Bl6J-kit^{W-v}/+]F1 − [W/W^{v}] mice), and congenic WBB6F1 normal mice were obtained from Jackson Laboratories (Bar Harbor, ME). All mice were bred and housed under pathogen-free conditions and maintained in the Biological Research Center at National Jewish Medical and Research Center. All protocols and experimental procedures were approved by the Institutional Animal Care and Use Committee of the National Jewish Medical and Research Center.

Experimental Protocol

Mice were exposed to O_3 at a concentration of 2.0 ppm for 3 hours. All parameters were measured 8 hours after completion of the O_3 exposure. For depletion of complement, 4 U of CVF (Naja naja kaouthia; Sigma, St. Louis, MO) was administered to the mice by intraperitoneal injection, 12 hours before O_3 exposure. Naja naja kaouthia inactivates C3–C9 (25). To prevent complement activation, 3 mg of mouse Crry–Ig (26) was administered by intraperitoneal injection to the mice 24 hours and just before O_3 exposure. As a control, rat IgG (Sigma) at the same dose and volume was injected intraperitoneally at the same time points. For depletion of neutrophils, 250 μg of RB6-8C5, a rat monoclonal antibody (27, 28) was administered to the mice by intraperitoneal injection 48 and 24 hours before O_3 exposure. Control mice received rat IgG as a control by intraperitoneal injection at 48 and 24 hours before O_3 exposure.

O_3 Exposure

Mice were exposed to O_3 at 2.0 ppm for 3 hours. They were placed in stainless steel wire cages set inside 240-L laminar flow inhalation chambers. High-efficiency particulate air–filtered room air was passed through these chambers at 25 changes/hour. The room temperature was maintained at 20 to 25°C. O_3 was generated by directing compressed medical-grade oxygen through an electrical discharge O_3 generator (Sander Ozonizer, Model 25; Erwin Sander Elektroapparatebau GmbH, Uetze-Eltze, Germany) located upstream of the exposure chamber. The O_3–air mixture was metered into the inlet air stream with

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mass flow controllers (Model #1359C; MKS Instruments Inc., Andover, MA). Simultaneous exposure to high-efficiency particulate air–filtered air was performed in a separate chamber with age- and treatment-matched control animals. O_3 concentrations were continuously monitored at mouse nose levels within the chamber with a photometric O_3 analyzer (Model 400A; Advanced Pollution Instrumentation Inc., San Diego, CA) and recorded on a strip-chart recorder. Calibration of the O_3 analyzer was performed by the Colorado Department of Public Health and Environment.

Determination of AHR

AHR was assessed as a change in airway resistance (cm H_2O/ml/second) after challenge with aerosolized methacholine (MCh). Anesthetized (pentobarbital sodium, 70–90 mg/kg, given intraperitoneally), tracheostomized (stainless steel cannula, 18 G) mice were mechanically ventilated, and lung function was assessed using methods described by Takeda and coworkers (29). Mice were placed in a ventilator (Model 683; Harvard Apparatus, South Natick, MA) with breathing controlled via a tracheostomy tube at 160 breaths/minute and a V_T of 150 μl with a positive end-expiratory pressure of 2 to 4 cm H_2O. Transpulmonary pressure, lung volume, and flow were determined. Lung resistance (R_L) was continuously computed (Labview; National Instruments, Dallas, TX) by fitting flow, volume, and pressure to an equation of motion. MCh aerosol was administered by nebulization of increasing concentrations (3–50 mg/ml). Maximum values of R_L were calculated and expressed.

Bronchoalveolar Lavage

Immediately after assessment of AHR and killing, lungs were lavaged via the tracheal tube with 1 ml of Hank's balanced salt solution (Gibco, Grand Island, NY). Total cell numbers were obtained (Coulter Counter Co., Hialeath, FL). Differential cell counts were performed by counting at least 200 cells on cytocentrifuged preparations in a blinded fashion (Model Cytospin 3; Shandon Ltd., Runcorn, Cheshire, UK) stained with Leukostat (Fisher Diagnostics, Fair Lawn, NJ).

Measurement of Cytokines and Total Protein in Bronchoalveolar Lavage Fluid

Cytokine levels in lung homogenates were determined by ELISA. Mouse interleukin (IL)-12, keratinocyte-derived chemokine (KC), and IL-1β (R&D Systems, Minneapolis, MN) were measured using commercial ELISA kits, per the manufacturers' instructions. TNF-α and macrophage inflammatory protein-2 (MIP-2) were measured with homemade kits using anti–TNF-α (R&D Systems; coating antibody, AFY10-NA; detection antibody, BAF410) and anti–MIP-2 antibody (R&D Systems; coating antibody, MAB452; detection antibody, BAF452). Detection limits for the assays were as follows: IL-12 (24 pg/ml), IL-1β (30 pg/ml), TNF-α (12 pg/ml), MIP-2 (7.5 pg/ml), and KC (18 pg/ml). For measurements in lung homogenates, lung tissue was frozen at −70°C immediately after killing. Lung tissue was mixed with a phosphate-buffered saline (PBS)–0.1% Triton-X100 solution containing proteinase inhibitors (PharMingen, San Diego, CA) at a 1:2.5 ratio of weight per volume. The specimens were homogenized and then centrifuged at 15,000 rpm for 15 minutes. The supernatants were frozen at −70°C until analysis. Total protein levels in bronchoalveolar lavage (BAL) fluid were measured by Lowry's method (30) using a Protein assay kit (Bio-Rad, Richmond, CA).

Assessment of C3 Levels in Serum and C3a Levels in BAL Fluid

C3 was measured by radial immunodiffusion using agarose containing a high-titered anti–human C3 antibody that cross-reacts with mouse C3 (intact and converted). A pool of normal mouse (C57BL/6 background) sera was used to generate a standard curve, and the results are reported as percent of the normal mouse serum pool.

C3a levels in BAL fluid were measured by ELISA (31). Briefly, microtiter plates were coated with a goat anti–rat IgG-Fc antibody (4.5 μg/ml in PBS, ICN Pharmaceuticals Inc., Cleveland, OH) for 2 hours. Then a monoclonal rat anti–mouse C3a antibody (1:100 dilution of hybridoma supernatant [clone 3/11] in blocking buffer) was added to each well. For detection, a polyclonal rabbit anti–mouse C3a antibody in blocking buffer was added and incubated for 1 hour, followed by a goat anti–rabbit IgG-Fc HRP-conjugated detection antibody (Bio-Rad). For quantification purposes, 100% CVF-activated C57BL/6j mouse plasma was used as a 50-μg/ml C3a standard.

Statistical Analysis

Data are presented as mean ± SEM. For analysis of the role of depletion or inhibition of complement in O_3-exposed mice, analysis of variance was used to determine the levels of difference between all groups. Comparisons for all pairs were performed using the Tukey–Kramer honest significant difference test (JMP 4.0; SAS Institute Inc, Cary, NC).

RESULTS

Depletion of Complement by CVF Prevents the Development of Airway Inflammation and Hyperresponsiveness

Exposure to O_3 leads to a number of acute changes in the airways, including a marked alteration in airway function and a significant inflammatory response. Between 8 and 24 hours after O_3 exposure, a threefold increase in levels of C3a was detected in BAL fluid (mean ± SEM: 3.18 ± 0.72 ng/50 μl) compared with mice exposed to air only (1.15 ± 0.72 ng/50 μl). When C57BL/6 mice were administered CVF, 12 hours before O_3 exposure, serum levels of C3 were no longer detectable at the time of assay, as shown in Figure 1A, in contrast to serum levels in mice exposed to either air or O_3 alone.

C57BL/6 mice are susceptible to O_3 exposure, and as shown in Figure 2, 8 hours after the 3-hour exposure was completed, the animals developed significant increases in airway resistance to inhaled MCh in a dose-dependent fashion. Mice depleted of complement with CVF showed significantly lower AHR throughout the MCh dose–response level.

In untreated mice, O_3 exposure resulted in a significant increase in total cell numbers in the BAL fluid, including neutrophils and, to a limited extent, eosinophils (Figure 3A). The decreases in AHR in CVF-treated mice were paralleled by significant reductions in total cell numbers and neutrophils (and eosinophils) in the BAL fluid (Figure 3A).

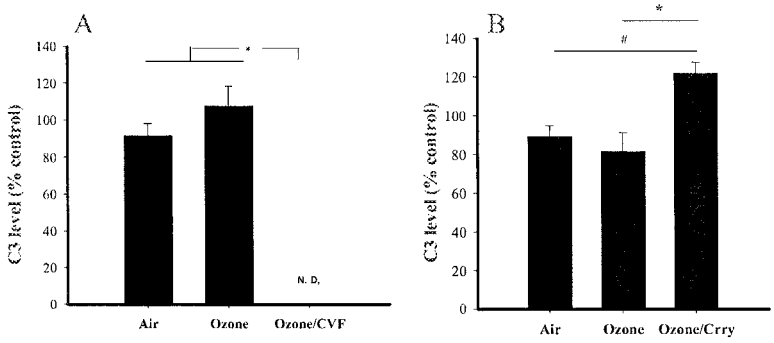

Figure 1. Serum C3 levels in mice after treatment with cobra venom factor (CVF) (*A*) or complement receptor–related gene y (Crry)–Ig (*B*). Mice were exposed to filtered air (for 3 hours) or ozone (O_3) (2 ppm for 3 hours) and treated with either CVF (O_3/CVF) or Crry–Ig (O_3/Crry–Ig) as described in METHODS. Each group consisted of 8 mice. *$p < 0.01$, #$p < 0.05$. N.D. = not detected.

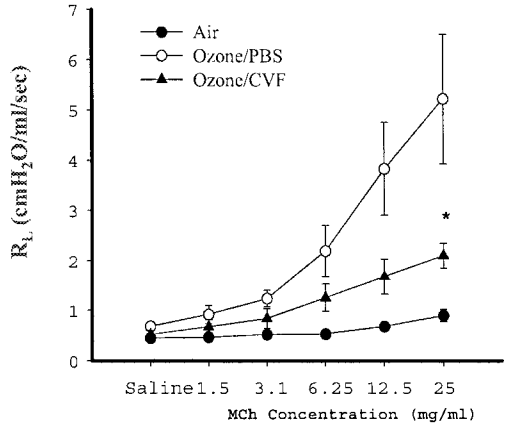

Figure 2. Depletion of complement by CVF attenuates the development of airway hyperresponsiveness (AHR) in response to acute O_3 exposure. Mice were treated with CVF or phosphate-buffered saline (PBS) (as described in METHODS) and were then exposed to filtered air or zone for 3 hours; 8 hours after completion of the exposures, airway function was assessed. AHR is expressed as airway resistance to inhaled methacholine (MCh) concentration. Each group consisted of 8 mice. *$p < 0.01$ compared with the O_3-exposed mice.

O_3 exposure also increased BAL fluid protein content when compared with air-exposed mice, and this increase was prevented by complement depletion with CVF (Figure 3B).

Crry–Ig Blocks the Development of O_3-induced Changes in Lung Function and Airway Inflammation

In addition to the approach using CVF for complement depletion, we also used Crry–Ig to prevent complement activation. As shown in Figure 1B, after treatment with Crry–Ig, O_3 exposure in fact resulted in a significant elevation of serum C3 levels, indicating the absence of complement activation. After treatment with Crry–Ig, the increase in serum C3 levels likely represents an overall decrease in C3 turnover, similar to what has been reported in Crry-overexpressing mice (32). As shown with CVF treatment, O_3-induced AHR was prevented if the mice were administered Crry–Ig before exposure (Figure 4). The decreases in AHR were throughout the MCh dose–response curve, and the extent of reduction was similar to that seen after CVF treatment. As shown with CVF, Crry–Ig markedly reduced the number of BAL neutrophils and eliminated the minor increases in BAL eosinophilia (Figure 5A); control (rat) IgG was without effect. BAL protein content was also significantly lower in Crry-Ig–treated mice (Figure 5B).

Effect of Crry–Ig on Production of Proneutrophil Cytokines and Chemokines

Accumulation of neutrophils in the lung in response to an insult is dependent on the release of a number of factors. Among them, IL-1β, TNF-α, MIP-2, and KC have been implicated. Acute O_3 exposure resulted in a significant increase in the levels of each of these proteins in lung homogenates (Figure 6). Pretreatment with Crry–Ig before O_3 exposure resulted in a significant decrease in each of these levels in lung homogenates. In contrast, levels of IL-12 were unaffected by O_3 exposure, and treatment with Crry–Ig was similarly without effect on IL-12 levels. Virtually identical results were observed when BAL fluid levels of these proteins were analyzed (data not shown).

Neutrophils Are Not Required for the Induction of AHR

On the basis of these data, it appeared that the development of AHR and neutrophilia may be associated, and both appeared dependent on complement activation. To directly determine the role of neutrophils in altered airway responsiveness, mice were administered a depleting antineutrophil antibody before O_3 exposure. The efficacy of this antibody treatment is illustrated in Figure 7A, demonstrating that the increase in BAL neutrophil numbers after O_3 exposure was virtually eliminated after antibody treatment. However, in the absence of an increase in BAL neutrophil numbers, AHR in response to O_3 exposure developed in an unimpaired fashion, with increased airway responsiveness virtually identical to the development in control mice, throughout the MCh dose–response curve (Figure 7B). Control Ig was without effect.

Development of AHR after O_3 Exposure Occurs in the Absence of Mast Cells

Complement-split products, especially the anaphylatoxins C3a and C5a, have been shown to be released from activated mast cells, and mast cells have been implicated in the response to O_3 (33). To assess the role of mast cells in the development of AHR after acute O_3 exposure in the present model, mast cell–deficient mice and their wild-type littermates were exposed to O_3 for 3 hours. After O_3 exposure, wild-type as well as mast cell–deficient mice developed a similar increase in responsiveness to MCh compared with air-exposed animals (Figure 8).

DISCUSSION

In studies linking complement activation, airway inflammation, and AHR, advantage has generally been taken of mice deficient in key components of the complement cascade or their receptors. The anaphylatoxins C3a or C5a are liberated after activation of the complement cascade and serve as proinflammatory mediators

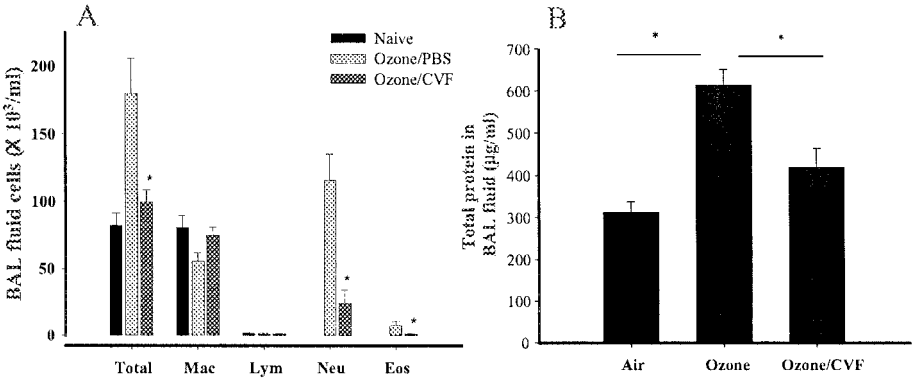

Figure 3. Depletion of complement by CVF decreases bronchoalveolar lavage (BAL) total cell, neutrophil, eosinophil numbers (*A*), and total protein levels (*B*) in O_3-exposed mice. BAL samples were obtained 8 hours after the exposures, at completion of the airway function measurements. The results are from the same groups of mice shown in Figure 2. *$p < 0.01$ compared with the O_3-exposed mice.

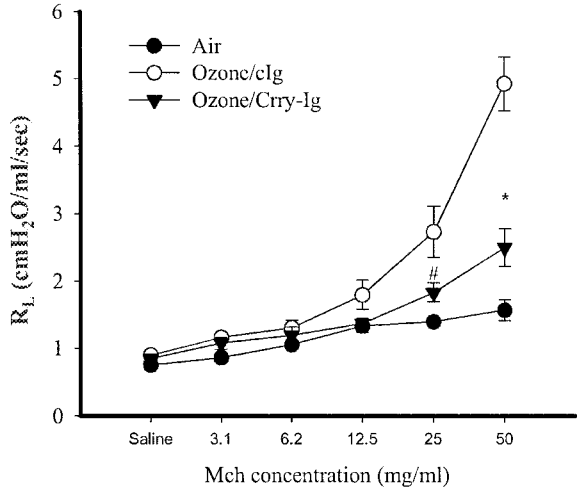

Figure 4. Inhibition of complement activation by Crry–Ig prevents the development of AHR to acute O_3 exposure. Mice received Crry–Ig or rat IgG (cIg) as a control (*see* METHODS) and were then exposed to filtered air or O_3 (2 ppm for 3 hours). Airway function was assessed 8 hours after completion of the exposures. Each group consisted of 8 mice. AHR is expressed as airway resistance to inhaled MCh. *$p < 0.01$ compared with the O_3-exposed mice.

through specific receptor binding on cell surfaces, including inflammatory cells and airway smooth muscle (34–40). Indeed, after O_3 exposure, increased levels of C3a could be detected in the BAL fluid, suggesting that one consequence of the exposure is complement activation. These results are similar to what has been described in BAL fluid of humans exposed to O_3 (24). In allergen-induced models of AHR, both C3 and the C3a receptors have been implicated in the development of altered airway responsiveness, cytokine production, and airway inflammation (15, 17, 18). In the case of C5, and the interaction between the active fragment C5a and its receptor, some controversy has emerged (16), but C5a, similar to C3a, appears to potentiate allergen-induced AHR (19). C5a has also been shown to trigger chemokine production, including neutrophil chemoattractants (34, 37, 38). Furthermore, C5a or the C5a receptor has been implicated in acute lung injury (40) and immune complex–induced AHR and airway inflammation (21). C3-deficient mice also did not develop increases in AHR induced by particulate matter (41).

In the present study, we used two approaches to study the role of complement activation in the O_3-induced development of AHR and inflammation. In one approach, complement was depleted after treatment with CVF. Such treatment has been shown to attenuate immune complex–induced responses within the lung (21). CVF, the reptile equivalent of C3b, binds to mammalian factor B, forming a stable CVFC3bBb complex and markedly depletes C3 as well as C5–C9 (25). The effectiveness of CVF treatment in the present study is supported by the marked depletion of serum C3 in the treated mice.

The second approach used Crry–Ig, a recombinant soluble form of the mouse membrane complement inhibitor Crry fused to the mouse IgG1 hinge, CH2 and CH3 domains (26, 42). Crry, complement receptor–related gene y, is a widely expressed membrane-bound intrinsic complement regulator protein. The murine homologs of human SCR1 are mouse CR1 and Crry. Crry–Ig exhibits decay-accelerating activities for both the classical and alternative pathways and stops the activation process at the C3 step. In addition, Crry demonstrates cofactor activity for the factor I–mediated cleavage of C3b (26). Crry–Ig has effectively prevented antibody-induced glomerulonephritis (26), antibody-induced fetal loss (43), and attenuated intestinal damage after ischemia/reperfusion injury (44). For CVF or Crry–Ig treatment, normal mice were targeted to avoid potential influences of genetic complement or complement receptor deficiency from a developmental point of view.

Acute O_3 exposure in C57BL/6 mice resulted in the rapid development of AHR and lung inflammation. The protocol using 2 ppm for 3 hours has been extensively characterized by Kleeberger and colleagues and is one in which a genetic susceptibility locus linked to TNF-α has been demonstrated (5, 8, 14). Eight hours after completion of the O_3 exposure, peak levels of increased resistance to inhaled MCh were seen, as well as a significant BAL neutrophilia and, to a lesser degree, eosinophilia. These changes were also accompanied by increases in levels of TNF-α, and the neutrophil chemoattractants MIP-2, KC, and IL-1β; levels of IL-12 were unchanged. After treatment with either CVF or Crry–Ig, virtually all of these responses were attenuated. AHR was significantly reduced, eosinophils in the BAL fluid were virtually absent, and BAL neutrophil numbers were reduced by more than 80%. In parallel, the levels of cytokines/chemokines in the lung homogenates and BAL fluid returned to near control levels, as did O_3-induced BAL protein levels.

All of the data implicate O_3-induced activation of the complement system in the initiation of these responses. In particular, the close association of reduction in AHR and neutrophil numbers (both not to baseline levels) suggested a potential causal relationship. To evaluate this possibility, mice were treated with a neutrophil-depleting antibody before O_3 exposure. This treatment was highly effective, depleting BAL neutrophils to an even greater extent than by preventing complement activation. Nonetheless,

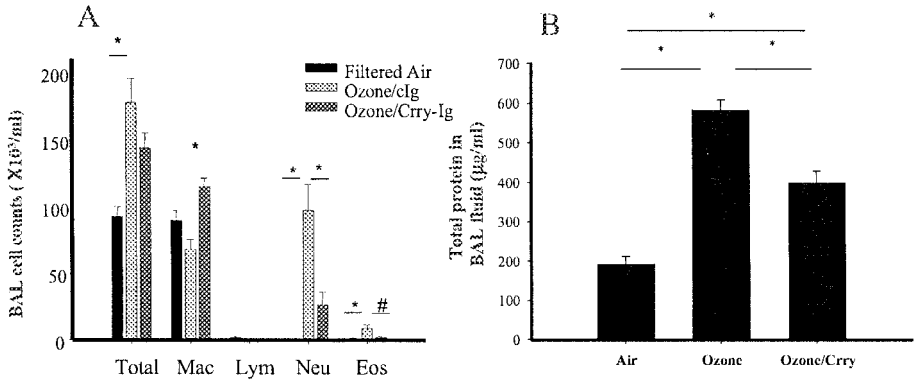

Figure 5. Inhibition of complement activation by Crry–Ig decreases BAL fluid neutrophilia, eosinophilia, and total protein levels in the O_3-exposed mice. The results are from the same groups of mice shown in Figure 4. *$p < 0.01$.

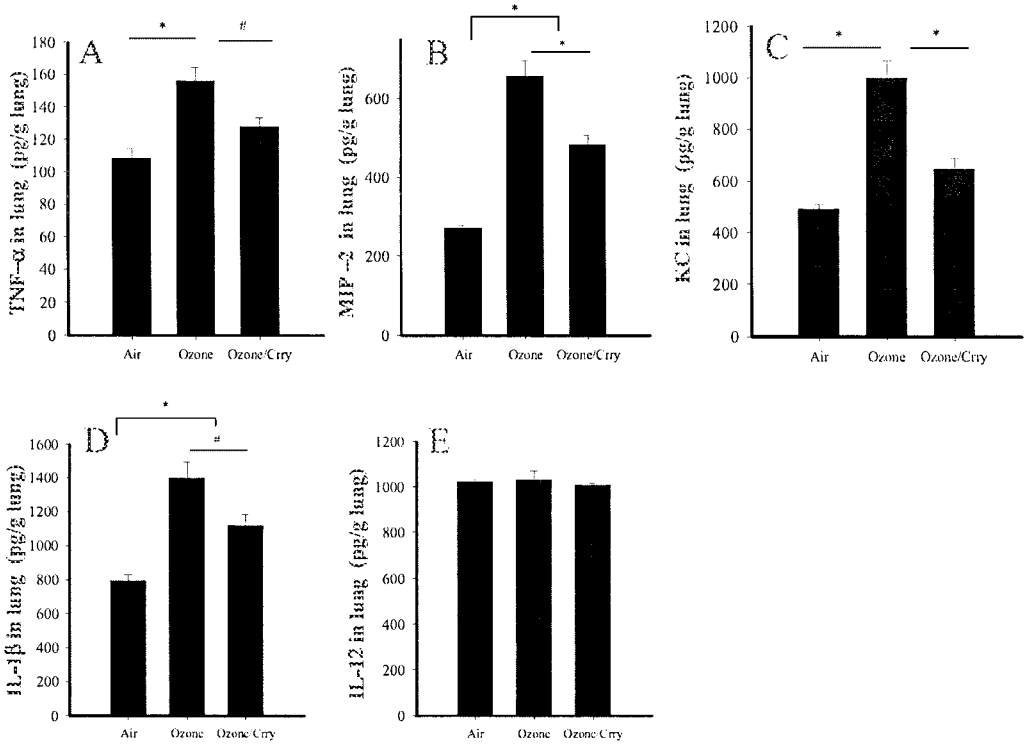

Figure 6. Cytokine levels in lung homogenates in mice treated with Crry–Ig. Tumor necrosis factor-α (TNF-α) (*A*), macrophage inflammatory protein-2 (MIP-2) (*B*), KC (*C*), interleukin (IL)-1β (*D*), and IL-12 (*E*) in lung homogenates. The results are from the same groups of mice shown in Figures 4 and 5. Each group consisted of 8 mice. *$p < 0.01$, #$p < 0.05$.

the development of O_3-induced AHR was unaffected, demonstrating the independence of the two processes. Complement-split products and especially C3a and C5a may activate mast cells, and in some studies mast cells have been implicated to at least partially mediate the influx of neutrophils into the lung after acute O_3 exposure (33, 45). However, other studies showed that the development of AHR after O_3 exposure is not dependent on mast cells (46). In the present study, mast cell–deficient mice developed the same degree of AHR after O_3 exposure as the congenic wild-type control mice, indicating that similar to the influx of neutrophils, mast cells are not essential to the development of AHR after O_3 exposure.

The use of CVF or Crry–Ig does not indicate the particular complement component involved in O_3-induced AHR or lung inflammation. There are several pathways through which O_3-induced AHR could develop in a complement activation–dependent manner. Acute O_3 exposure is known to primarily target epithelial cells. Epithelial cells are known to be sources of both C3 and C5 (22, 23, 41). Epithelial cell injury may trigger complement convertase activation, liberating C3a and/or C5a from injured or necrotic epithelial cells. Complement activation may also occur through reactions with injured cells, resulting in production of C3a and C5a. In turn, as airway smooth muscle expresses both C3a and C5a receptors (39), bronchoconstriction to inhaled MCh may ensue. In a more indirect manner, O_3 may activate complement via increases in reactive oxygen species (47, 48). Interestingly, in humans exposed to O_3, C3a levels were increased (24, 49). Both C3a and C5a are also known chemoattractants for inflammatory cells (34, 50). Acute O_3 exposure leads to the production of the early-response cytokines,

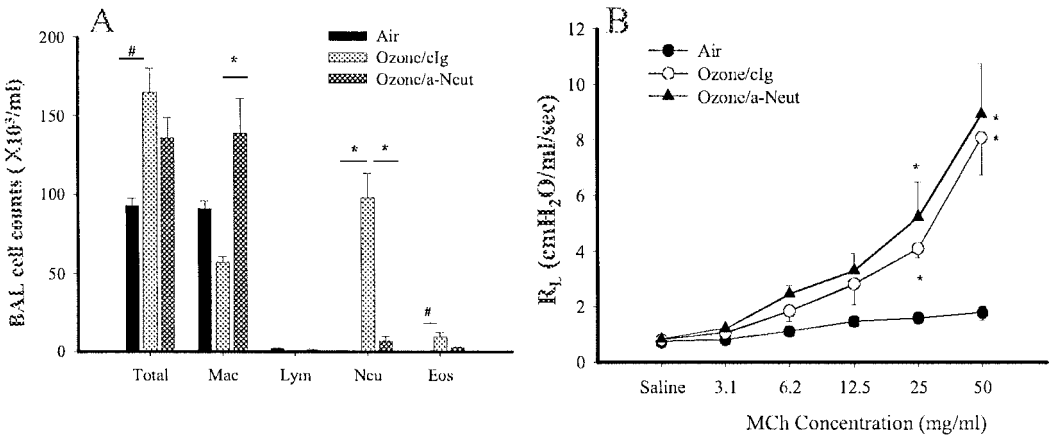

Figure 7. Depletion of neutrophils by RB6-8C5 monoclonal antibody (mAb) does not affect the development of AHR in response to O_3 exposure. As described in METHODS, mice were administered mAb or rat IgG (cIg) before the exposure to filtered air or O_3. Eight hours after completion of the exposures, airway function was monitored and BAL samples were obtained. (*A*) BAL inflammatory cell composition. (*B*) Airway resistance to inhaled MCh. Each group consisted of 8 mice. *$p < 0.01$, #$p < 0.05$. In (*B*), *$p < 0.01$ compared with the air-exposed group.

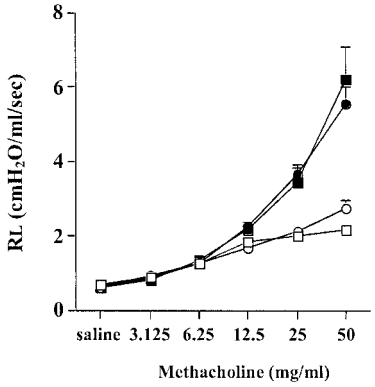

Figure 8. Mast cell–deficient mice develop the same degree of AHR after acute O_3 exposure as control mice. Mast cell–deficient mice (W/W^v) and congenic wild-type control mice (+/+) were exposed to O_3 (*filled circles* and *filled squares*, respectively) and or air (*open circles* and *open squares*, respectively) (n = 8 in each group). After O_3 exposure, +/+ and W/W^v mice developed a similar degree of airway reactivity to MCh. *$p < 0.05$ compared with +/+ air and W/W^v air. Mean ± SEM are given.

TNF-α and IL-1β, as well as the macrophage-derived neutrophil chemokines, MIP-2 and KC. Neutrophil accumulation in the BAL fluid is one of the earliest events after O_3 exposure. Treatment with either CVF or Crry–Ig significantly reduced BAL and lung homogenate levels of both cytokines and chemokines likely accounting for the decreases in neutrophil numbers. IL-12 levels were unaffected by O_3 exposure and were not altered by treatment with CVF or Crry–Ig. These apparently independent effects of C3a/C5a on airway smooth muscle and inflammatory cell accumulation could explain the dissociation observed between AHR and neutrophil infiltration.

Despite all of the supportive data pointing to the prevention of C3 and/or C5 activation by CVF or Crry–Ig, it is possible that the effects of these complement activation inhibitors are not solely on C3- or C5-mediated responses but regulate other complement-dependent pathways. In complement-sufficient mice, CVF attenuated the AHR response and reduced histamine levels in an immune complex–driven model but was surprisingly ineffective in C5-deficient mice (21). Whether a compensatory and CVF-resistant pathway emerged to dominate in the C5-deficient mice is unclear.

In summary, these studies suggest that the very rapid development of AHR to inhaled MCh and the accompanying neutrophil-dominated inflammatory response, which follows acute O_3 exposure, is the result of complement activation. AHR is directly induced by complementary activation but independent of mast cell and neutrophil accumulation. In many ways, the data support a common mechanism for the development of altered airway responsiveness, a pathway dependent on complement activation that may be shared by allergen, immune complexes, and environmental pollutants.

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