



CXC chemokines modulate IgE secretion and pulmonary inflammation in a model of allergic asthma

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Abstract

The pathophysiology of asthma is influenced by exposure to allergens and endotoxin. Although the role of allergen-induced eosinophilia has been widely studied, neutrophil-mediated responses remain elusive. A role for neutrophils in the asthmatic responses is likely since human neutrophils have been shown to express IgE receptors, as well as receptors for many cytokines and chemokines implicated in the pathogenesis of asthma. In this study we investigated neutrophil involvement in a novel, house dust extract (HDE) induced model of asthma-like pulmonary inflammation. Mice were immunized and challenged with HDE containing high levels of cockroach allergens, 377 U/ml Bla g1 and 6249 ng/ml Bla g2. The biological activity of the murine chemokines KC and MIP-2 was inhibited with specific rabbit antisera. Differential counting of cells recovered from the bronchoalveolar lavage (BAL) fluid showed that neutralization of KC and MIP-2 significantly decreased pulmonary recruitment of neutrophils (reduced 86%) and lymphocytes (reduced 76%). Neutralization of these chemokines also exerted a systemic effect with a significant decrease in plasma IgE levels, 547 ng/ml $\pm$ 65 compared to 1314 ng/ml $\pm$ 247 for control sera treated animals. This study shows that CXC chemokines play an important role in allergy and asthma both at the level of pulmonary cell recruitment and systemic immune responses.

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1. Introduction

Although asthma has long been thought of as an eosinophilic disease of the airways, the involvement of neutrophils in disease pathology is likely. A role for neutrophils has been suggested from studies analyzing the sputum of asthmatic patients that showed neutrophils to be the major cell type present in the sputum of patients with asthma exacerbations, as well as subjects with severe asthma [1,2]. In addition to the presence of neutrophils in severe asthma and asthma exacerbations,

it has recently been shown that the occurrence of non-eosinophilic asthma is far more common than previously believed not only in severe asthma, but also in mild and moderate asthma [3].

In accordance with the increases of neutrophils seen in asthmatic patients there have been various studies showing increased levels of CXCL8/IL-8 in the BAL fluid of asthmatics [4–6]. Furthermore, the levels of CXCL8 correlate with the percentage of neutrophils present [4–6]. While CXCL8 has not been identified in the mouse, the CXC chemokines KC and MIP-2 are CXCL8 homologs believed to be important in the trafficking of neutrophils. Administration of KC and MIP-2 neutralizing antibodies prior to antigen challenge in a murine OVA model of pulmonary lung inflammation led to a significant decrease in BAL fluid neutrophil levels compared to animals treated with an isotype control antibody [7].

We have established a novel murine model of asthma-like pulmonary inflammation utilizing a house dust extract

Abbreviations: AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; CRA, cockroach allergen; HDE, house dust extract; MCh, acetyl β -methylcholine; Penh, enhanced pause; PI, pulmonary inflammation.

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prepared from dust collected from the homes of asthmatic children that contains high levels of the cockroach allergens Bla g1 and Bla g2. A heterogeneous inflammatory response consisting of neutrophils, lymphocytes and eosinophils is elicited following immunization and subsequent challenge [8,9]. In order to determine whether KC and MIP-2 are important in neutrophil chemotaxis in our model, KC and MIP-2 neutralizing antisera were administered prior to intratracheal challenge of the house dust extract. We report that the neutralization of KC and MIP-2 does not decrease airway hyperresponsiveness although there are significant decreases in plasma IgE levels and BAL fluid neutrophil, and lymphocyte cell counts. These data support a novel role for CXCL8 homologs in adaptive immune responses and suggest distinct mechanisms of pulmonary inflammation from airway hyperresponsiveness in our model of allergic inflammation.

2. Materials and methods

2.1. Animals

Female BALB/c mice were obtained from Harlan Sprague–Dawley (Indianapolis, IN) and kept under standard laboratory conditions. Mice were housed in a temperature-controlled room with a 12-h light/dark cycle and allowed food and water ad libitum. All experiments have been approved by the University of Michigan Animal Use Committee.

2.2. House dust collection and extraction

Dust was collected from a 1-m² area of the kitchen from 10 homes of asthmatic children in Detroit, MI using an electric vacuum cleaner with a dust collector (Indoor Biotechnologies, Charlottesville, VA) as previously described [8]. The house dust extract was prepared by adding 2 ml of sterile PBS to each sample and mixing the samples overnight at 4 °C on a rotator. Samples were then centrifuged for 10 min at 1000 × g, 4 °C, the supernatant was collected, recentrifuged and the supernatant was used for analysis of various indoor allergens and endotoxin as previously described [8]. The home with the highest content of cockroach allergen was revisited to obtain a larger sample of house dust. This subsequent collection was extracted in 30 ml of PBS, processed as before and allergen content was again determined. Aliquots of house dust extract (HDE) from this second collection were stored at –70 °C to be used in all experiments. The final collection of HDE was found to contain 270 pg/ml endotoxin and 377 U/ml of the cockroach allergen Bla g1 and 6249 ng/ml of Bla g2 with undetectable levels of dust mite allergens [8].

2.3. Antisera preparation

Rabbit anti-mouse MIP-2 and goat anti-mouse KC were prepared as described [10]. These antisera are specific for MIP-2 and KC, respectively, and neutralize biological activity. These antisera have been shown to have specificity for the

individual chemokines [11] and have been shown to neutralize their respective chemotactic activities in vivo [10,12,13].

2.4. Animals and induction of asthma

The house dust extract was diluted 1:10 in sterile PBS immediately prior to use, emulsified in TiterMax Gold adjuvant (1:1, CytRx, Norcross, GA) and 100 µl per mouse of the HDE:adjuvant was administered intraperitoneally (i.p.) on day 0. On days 14 and 21 mice were treated with 1 ml of antisera or control serum by subcutaneous injection. The anti-chemokine antisera were either used alone or in combination, as indicated in the figure legends. Two hours after serum injections mice were anesthetized using isoflurane (Baxter, Deerfield, IL) and challenged intratracheally (i.t.) with 1:10 diluted HDE, as described [8]. Briefly, the anesthetized mouse was suspended from the front incisors on an inclined board and the base of the tail was taped to support its weight. While holding the jaw open the tongue was extended using forceps and two 25-µl aliquots of diluted HDE were pipetted at the base of the oropharynx. On day 22, airway hyperresponsiveness was measured. On day 23, mice were sacrificed by cervical dislocation and plasma, BAL fluid and lungs were collected.

2.5. Airway hyperresponsiveness

Airway hyperresponsiveness (AHR) of mice was measured 24 h after the last airway challenge using a whole body plethysmography system (Buxco, Troy, NY). Mice were placed in the main chamber and allowed to acclimatize for 10 min. Baseline values were then recorded for 5 min. Mice were subsequently challenged with aerosolized PBS or increasing doses of aerosolized β-methylcholine (MCh; Sigma, St. Louis, MO) for a 2-min period. The response was recorded for 5 min after each dose by measuring the pressure differences between the main chamber containing the mouse and a reference chamber. Differences in signals quantified during respiratory cycle are reflected by enhanced pause (Penh). Penh values are normalized as a percent increase of average Penh for each MCh dose over the average Penh for PBS challenge. Penh is widely used in models of pulmonary dysfunction and has been shown to correlate with airway resistance [14]. Although there is controversy about whether Penh reflects airways hyperreactivity as opposed to nasal passages, the Penh measurements have been documented to correlate closely with invasive measurements [15]. Calculating the change in Penh will account for pressure changes resultant from heating and humidification of air traveling between the chamber and lungs, termed gas conditioning. If gas conditioning is present, then an absolute Penh may not be indicative of bronchial constriction [16]. Our experimental design ensures that the Penh does reflect increases in bronchial constriction since both the control antisera mice and the antichemokine-treated mice are assessed on the same day and time under identical conditions, which would negate any effect of gas conditioning.

2.6. Peripheral blood analysis

For blood counting, 20 μ l of EDTA (Sigma) anti-coagulated blood was collected from the tail 48 h after the second airway challenge while the mice were anesthetized with ketamine and xylazine solutions (87 μ g/g ketamine plus 13 μ g/g xylazine, Ketaset[®]; Fort Dodge Laboratories, Inc., Fort Dodge, IA. Rompun[®]; Bayer Corporation, Shawnee Mission, KS). For a complete blood count, a Hemavet Mascot Multispecies Hematology System Counter 1500R (CDC Technologies Inc., Oxford, CT) was used [17].

Subsequently blood was also collected from the retro-orbital venous plexus into tubes containing 50 μ l of 169 mM EDTA before the mice were sacrificed. Plasma was collected by centrifugation (600 $\times$ g, 5 min) and stored at -20°C for later chemokine and IgE analyses.

2.7. BAL fluid analysis

Following exsanguination, the trachea was exposed and intubated with a polyethylene catheter. Lungs were lavaged with Hanks' balanced salt solution (Gibco, Grand Island, NY) and two 1-ml fractions were collected. The two lavages were centrifuged at 1000 $\times$ g for 5 min. The supernatant of the first wash was saved for later cytokine and chemokine analyses while the supernatant of the second 1-ml fraction was discarded. The cell pellets from both washes were combined and total cells were counted using a Coulter counter model ZF (Coulter Electronics, Hialeah, FL). Cytospin slides were prepared by diluting 100,000 cells in 200 μ l cytospin fluid; slides were stained with Diff-Quick (Baxter, Detroit, MI) and differentials calculated from counting of 300 cells. The number of individual cell types was calculated for each mouse by multiplying the percentage of cells by the total cell count and the data are expressed as cells/mouse. The data thus represent the total number of cells recovered from the lung, which makes comparisons between different models easier since one does not need to calculate dilution factors between separate reports.

2.8. Myeloperoxidase assay

After the BAL fluid was collected, the right lung was removed and immediately placed in 3 ml of ice-cold homogenization buffer containing 0.05% Triton X-100 (Sigma) in PBS. Three 10-s rounds of homogenization and sonication were followed by centrifugation at 15,000 $\times$ g for 15 min at 4°C . For the myeloperoxidase (MPO) assay 40 μ l of sample supernatant was added in duplicate to a 96-well plate and 200 μ l assay buffer containing 100 mM potassium phosphate, 0.834 ml *o*-dianisidine HCl (10 mg/ml; Sigma) and 0.083 ml 0.3% H_2O_2 in 50 ml deionized water was added immediately prior to reading. The reaction was assayed in a Biotek microplate reader (Bio-Tek Instruments, Inc., Winooski, VT) every 10 s at 465 nm. The data are expressed as the slope of change in optical density over 100 s.

2.9. Cytokine and IgE ELISAs

Ninety-six-well plates (Nunc Immunoplate Maxisorb; Nunc, Neptune, NJ) were coated with antibody diluted in PBS overnight at 4°C (KC or MIP-2 rat monoclonal antibodies, or eotaxin or MCP-1 goat polyclonal antibodies, all from R&D Systems, Minneapolis, MN; IgE rat MAb – BD Pharmingen, San Diego, CA). Plates were washed five times in wash buffer containing 0.05% Tween 20 (FisherBiotech, Fair Lawn, NJ) in PBS and blocked with Blotto blocking solution (Pierce, Rockford, IL) on a shaker for 1 h at room temperature. Standards and plasma were diluted in dilution buffer containing 10% Blotto, 0.05% Tween 20 and 0.0001% BSA in $1\times$ PBS, added to plates and incubated at room temperature for 2 h on a shaker. Plates were washed and secondary antibodies diluted in dilution buffer were added (KC, MIP-2, MCP or eotaxin goat polyclonal Ab – R&D Systems, Minneapolis, MN; IgE rat polyclonal Ab – BioSource international, Camarillo, CA). Plates were incubated for 2 h at room temperature then washed as before. HRP-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA) was added and plates were incubated for 1 h at room temperature. Color was developed with 3,3',5,5'-tetramethylbenzidine and the reaction was stopped with 1.5 N H_2SO_4 . Plates were read at 465 and 590 nm in a microplate reader (Bio-Tek Instruments, Winooski, VT). The lower limit of sensitivity for the assays was typically <10 pg/ml, and samples frequently needed to be diluted in order to accurately measure the cytokines. The manufacturer of the antibodies has extensively tested the specificity of the individual antibodies.

2.10. Statistics

Values are expressed as mean $\pm$ SEM. GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA) was used to calculate *p* values using unpaired Student's *t*-test.

3. Results

3.1. BAL fluid levels of KC and MIP-2 are increased following house dust extract challenge

The inflammatory response in a novel model of asthma-like pulmonary inflammation following sensitization and challenge with HDE containing high levels of cockroach allergen has been described [8]. Airway hyperresponsiveness to methacholine, as measured by a whole body plethysmograph system (Buxco, Troy, NY), peaks at 24 h following the final challenge. BAL fluid eosinophils, lymphocytes and neutrophils are significantly increased following sensitization and challenge with HDE. Pulmonary neutrophil influx was observed as early as 4 h post-final i.t. challenge and remained elevated throughout the 48 h following the last challenge. Pulmonary eosinophil and lymphocyte influx are observed by 36 h and peaks at 48 h. A marked increase in plasma IgE, MCP-1 and eotaxin at 48 h is observed in this model as well [8,9,18].

In the present study, mice immunized intraperitoneally on day 0 with house dust extract and adjuvant were subsequently challenged intratracheally on days 14 and 21. Mice were sacrificed at several early time points following the final challenge and BAL fluid levels of the CXC chemokines KC and MIP-2 were assessed by ELISA in order to characterize the early response to challenge. KC and MIP-2 peaked in the BAL fluid by 2 h after the final challenge and quickly fell below the lower limit of detection by 8 h (Fig. 1).

3.2. Individual neutralization of KC or MIP-2 fails to decrease pulmonary cell recruitment in our model of HDE-induced allergic responses

In order to determine the role of KC and MIP-2 in our model mice were treated with 1 ml of control sera or antisera consisting of antibodies to KC or MIP-2 individually or in combination. Experiments were performed using antisera which have been shown to neutralize chemotactic activity in vivo [10,12,13]. To determine potential effects of KC or MIP-2 individually, either KC or MIP-2 antisera were administered singly prior to pulmonary challenge. Neither KC nor MIP-2 antisera treatment prior to challenge had any effect on pulmonary cell infiltration, myeloperoxidase activity, airway hyperresponsiveness, total plasma IgE or CC chemokine levels (data not shown). Given the redundancy in the CXC chemokines, these results were not surprising.

3.3. Neutralization of both KC and MIP-2 reduces pulmonary inflammation

Immunization and challenge with HDE alters the profile of circulating leukocytes with an increase in white blood cells compared to naïve animals (data not shown). The current studies were designed to reduce inflammatory cell recruitment to the site of local inflammation, but we also verified that antibody treatment was not toxic to circulating cells. As expected, neutralization of KC and MIP-2 did not alter the circulating cell populations compared to mice treated with control

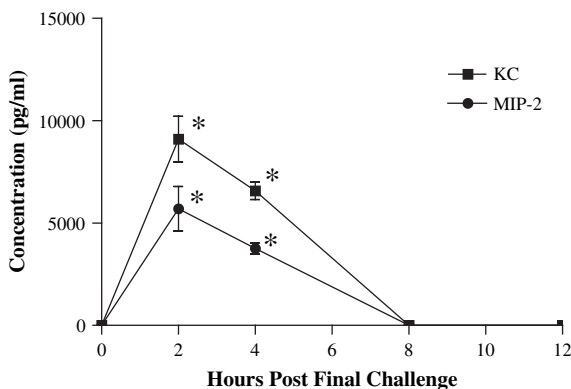


Fig. 1. BAL chemokine levels. BAL fluid KC and MIP-2 levels were assessed at the indicated time points following the final pulmonary challenge. 0-, 8- and 12-h time points were below the lower limit of detection (<10 pg/ml). Each value is the mean $\pm$ SEM for $n = 4$ mice. * = Significantly different from time 0.

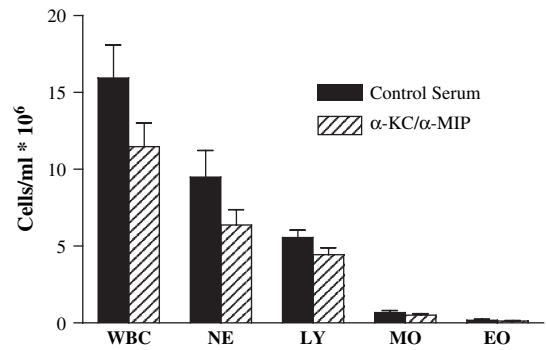


Fig. 2. Circulating white cell counts. A total and differential white cell count was performed immediately prior to sacrifice. WBC, white blood cells; NE, neutrophils; LY, lymphocytes; MO, monocytes; and EO, eosinophils. Each value is the mean $\pm$ SEM for $n = 7$ mice in two experiments, $p > 0.05$.

antibody (Fig. 2). Lung myeloperoxidase activity, which reflects the total granulocyte sequestration within the lung, was only slightly reduced in animals when KC and MIP-2 were neutralized prior to pulmonary challenge compared to controls (Fig. 3). This reduction was not statistically significant. It should be noted that the MPO activity was determined from lungs that had previously been lavaged to harvest the intra-alveolar cells, including the recruited inflammatory cells; therefore, the pulmonary MPO activity represents neutrophils sequestered within the interstitium and not those recruited into the alveolar space.

Combined treatment with anti-KC and anti-MIP-2 resulted in a 33% reduction in total cellular influx to the airway compared to control animals (data not shown). Since the antisera were specific for the neutrophil chemoattractants KC and MIP-2, it was anticipated that there would be a significant reduction in the number of neutrophils recruited to the lung in response to the HDE. As expected, pulmonary neutrophil levels were decreased by 86% in mice treated with anti-KC plus anti-MIP-2 prior to challenge (Fig. 4A). This reduction in the number of recruited neutrophils was statistically significant. BAL fluid lymphocyte and eosinophil counts were both reduced as a result of KC and MIP-2 blockade as well (76% and 68% reduction, Fig. 4B and C, respectively). The reduction in the number of lymphocytes was statistically significant and the decrease in the number of eosinophils approached

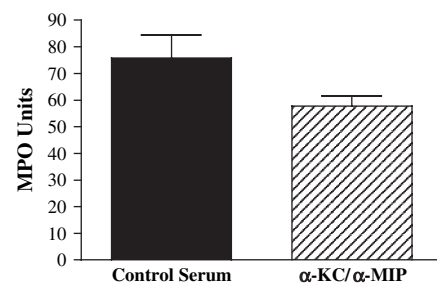


Fig. 3. Lung myeloperoxidase levels. Myeloperoxidase levels of whole lung homogenates were measured 48 h after the final pulmonary challenge. The MPO levels were measured after the lungs were lavaged to harvest bronchoalveolar cells. Each value is the mean $\pm$ SEM for $n = 10$ mice from three separate experiments.

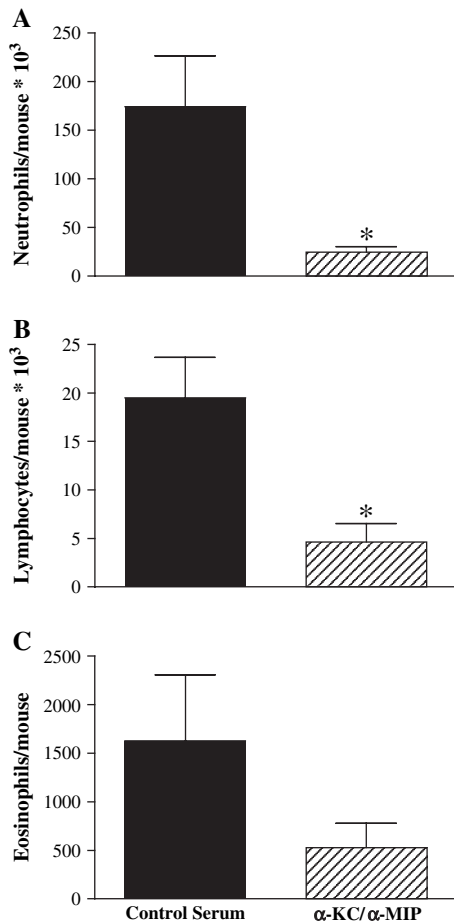


Fig. 4. Profile of cells recovered from BAL fluid 48 h after the final pulmonary challenge. Cytospins were prepared and a differential performed by counting of 300 cells. (A) Neutrophils, * $p = 0.0081$, control serum vs α -KC plus α -MIP-2; (B) lymphocytes, * $p = 0.0037$, control serum vs α -KC plus α -MIP-2; and (C) eosinophils, differences not significant. Each value is the mean $\pm$ SEM for $n = 10$ mice from three separate experiments.

significance ($p = 0.004$ for lymphocytes and 0.134 for eosinophils).

Immunization and challenge with HDE results in a dose-dependent increase in airway responsiveness to MCh challenge as determined by a whole body plethysmography [8,9,18]. Treating mice with the combination anti-KC and anti-MIP-2 antisera prior to i.t. challenge failed to attenuate the airway hyperresponsiveness seen in our model; in fact the two lines are virtually superimposable (Fig. 5). These increases in Penh are similar to those previously reported with this model [8,9,18].

CC chemokines are important eosinophil and lymphocyte chemoattractants in clinical asthma [19,20], as well as in our model system [8]. In order to determine if neutralization of CXC chemokines alters the plasma levels of the CC chemokines, eotaxin and MCP-1 levels were assessed at the time of sacrifice. The chemokine levels in the BAL were below the limit of detection (<10 pg/ml). Plasma levels of the chemokines were detectable, and there were no significant differences in the levels of eotaxin or MCP-1 in mice receiving antisera compared to those treated with control sera prior to challenge (Fig. 6A and B). These data help to show that the

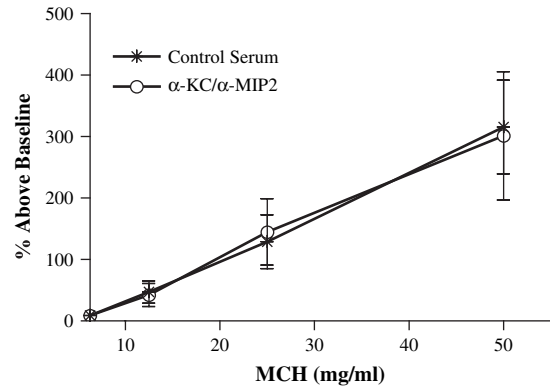


Fig. 5. Airway hyperresponsiveness. AHR was assessed 24 h after the last pulmonary challenge. Values are reported as a percent increase in the average Penh of each dose above the average Penh following a PBS challenge. Each value is the mean $\pm$ SEM for $n = 10$ mice from three separate experiments.

antibodies have specificity for KC and MIP-2 and do not cross-react with these CC chemokines. KC and MIP-2 levels were not measured at this time since they peak at 2 h and fall below the lower limit of detection by 8-h post-pulmonary challenge (Fig. 1).

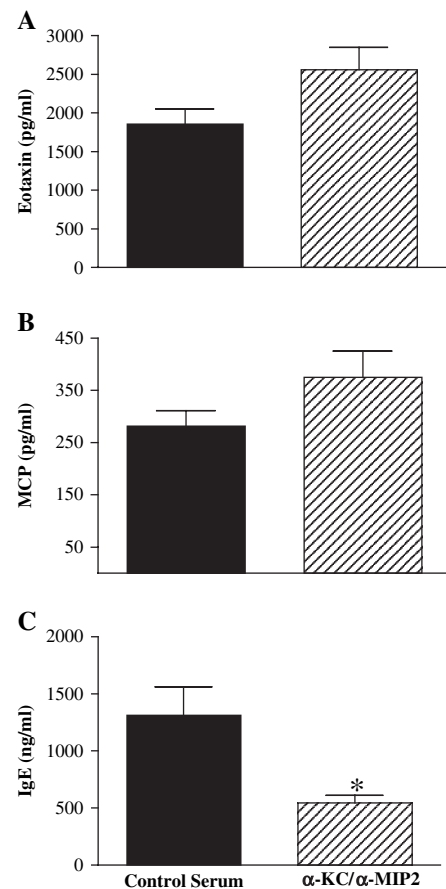


Fig. 6. Plasma levels of eotaxin, MCP, and IgE. BAL fluid cytokine and IgE levels were below the lower limit of detection for both treatment groups, 78 pg/ml, 5 pg/ml, 2.4 ng/ml for eotaxin, MCP-1 and IgE, respectively (data not shown). (A) Eotaxin levels, no significant difference between the groups. (B) MCP levels, no significant difference between the groups. (C) IgE levels, $p = 0.0076$ for total plasma IgE. Each value is the mean $\pm$ SEM for $n = 10$ mice from three separate experiments.

Total plasma IgE was also measured by ELISA. Surprisingly, mice treated with anti-KC and anti-MIP-2 antisera have attenuated IgE secretion (Fig. 6C). These results are interesting in that antisera were only administered on days 14 and 21 prior to challenge and not at time of sensitization, indicating that the neutralization of CXC chemokines was able to attenuate the systemic allergic response. There was no reduction in total plasma IgG (data not shown), indicating that the chemokine inhibition exhibited a specific effect in this model.

4. Discussion

We investigated the role of the CXC chemokines KC and MIP-2 in the pulmonary cell infiltration and airway hyperresponsiveness observed in a murine model of house dust extract-induced pulmonary inflammation. The major findings of this study were that neutralization of KC and MIP-2 not only attenuated neutrophil pulmonary influx but also infiltration of lymphocytes and, to a lesser extent, eosinophils. This treatment also reduced the circulating levels of plasma IgE, indicating modulation of both the local and systemic responses by CXC chemokines.

Despite the marked decrease in inflammatory cell recruitment to the lung, airway hyperresponsiveness to methylcholine challenge was unaltered in mice treated with combined anti-KC and anti-MIP-2 antisera. The mechanism of airway hyperresponsiveness in lung disease remains unclear but various studies have shown that airway hyperresponsiveness to methylcholine is independent of pulmonary cell infiltration. Studies using B-cell deficient mice have shown airway hyperresponsiveness similar to wild-type animals following immunization and airway challenge with ovalbumin [21]. Tournay et al. have shown that airway hyperresponsiveness occurs independently of airway eosinophilia in a house dust mite allergen model of antigen-specific pulmonary inflammation [22]. Previous work with our model is in agreement with these studies in that airway hyperresponsiveness peaks at 24 h following the final HDE challenge while eosinophil and lymphocyte recruitment to the lung is not seen until 36 h and peaks at 48 h in BALB/c mice [8], suggesting that these cell populations are not required to elicit responsiveness.

While few studies have investigated the role neutrophils play in airway hyperresponsiveness Takaoka et al. showed that treating mice with an antibody to human IL-8R which cross-reacts with mouse CXCR2 prior to ovalbumin challenge resulted in near complete attenuation of both neutrophil influx and airway hyperresponsiveness [23]. Conversely, Lefort et al. have shown that airway hyperresponsiveness is not dependent on neutrophil recruitment to the lung following systemic administration of LPS. In this study bronchial hyperresponsiveness was unaltered in mice pretreated with an anti-granulocyte antibody that depleted the animals of circulating neutrophils [24]. We report that HDE-induced airway hyperresponsiveness is not altered by neutralization of the CXCL8 homologs KC and MIP-2 despite the marked decrease in neutrophil influx to the airways. In our model of pulmonary inflammation neutrophils represent a major infiltrating cell

population. Neutrophils are recruited early, peak by 24 h and remain elevated through 48 h following the last pulmonary challenge [8]. CXC chemokines, such as MIP-2, KC, GRO, ENA-78 and LIX, are the predominant chemokines involved in neutrophil chemotaxis. CXCR2 present on the neutrophil is the receptor for all ELR⁺ CXC chemokines. In the present study neutralization of KC and MIP-2 resulted in an 86% decrease in neutrophils in the BAL fluid, however, the total number of neutrophils present in the airway still averaged over 120,000 cells/ml and constituted 17% of the total cells leading to the possibility that we did not inhibit neutrophil chemotaxis sufficiently to decrease airway hyperresponsiveness.

In addition to the expected decrease in pulmonary neutrophil recruitment BAL fluid lymphocyte levels were also reduced. Whether the reduction of lymphocytes and eosinophils in the BAL fluid results directly from KC and MIP-2 neutralization or is an indirect effect of the treatment has yet to be established in our studies. The decrease of lymphocytes we observe in the BAL fluid from mice treated with anti-KC and anti-MIP-2 sera may be indirectly caused by the marked decrease in neutrophil influx. Studies by Morita et al. have shown that neutralization of KC, with the same antisera used in our studies, resulted in prolonged survival of heart allografts and decreased lymphocyte infiltration [12]. Further studies by the same group showed that depleting animals of neutrophils prolonged allograft survival indicating a link between the innate and adaptive immune responses in vivo. CXCL8 may function to activate neutrophils to release granules that are chemotactic for lymphocytes [25]. In vitro neutrophils enhance eosinophil chemotaxis by inactivating C5a in the upper compartment and thus maintaining a steep C5a chemotactic gradient [26]. It is possible that the effects of combined pretreatment with anti-KC plus anti-MIP-2 serum seen in the present studies are indirectly mediated by the decrease in pulmonary neutrophils. Alternatively, KC and MIP-2 may be directly mediating eosinophil and lymphocyte chemotaxis.

Some studies indicate that CXCL8 is an important mediator of eosinophil chemotaxis [27,28] while others failed to show eosinophil chemotaxis in response to CXCL8 and lack of CXCR1 or 2 expression on eosinophils [29]. Lampinen et al. have shown that eosinophils isolated from asthmatic patients but not healthy controls are chemotactic to CXCL8 [30] suggesting altered receptor expression on eosinophils from asthmatic subjects. The differential findings concerning the direct effects of CXCL8 on eosinophil chemotaxis may be explained by neutrophil contamination of cultures [29].

Lymphocytes isolated from human peripheral blood have been shown to express CXCR1 and 2 [31] and both in vitro and in vivo studies have consistently shown that CXCL8 can induce lymphocyte chemotaxis [25,31–34]. Schratzberger et al. report that both T and B lymphocytes chemotax to CXCL8 [35]. In contrast to these findings De Sanctis et al. have shown that IL-8r^{-/-} mice that have a targeted deletion of the IL-8 receptor homolog have increased lymphocyte recruitment to the lung and increased IgE in an OVA-model of asthma-like pulmonary inflammation. The increased lymphocytes in these studies were primarily B lymphocytes [36].

We have shown that neutralization of KC and MIP-2 prior to HDE challenge led to decreased circulating levels of IgE. To the best of our knowledge this is a novel finding that has not been previously reported. Studies by Shim et al. have shown that IL-13-induced leukocyte recruitment and mucin production can be inhibited by treating rats with anti-IL-8 antibodies prior to IL-13 instillation to the lung, however, these studies did not address IgE levels [37]. A number of studies have shown that the Th2 cytokines IL-4 and IL-13 can induce CXCL8 production by mast cells and monocytes [38,39]. Furthermore, the expression of CXCL8 receptors CXCR1 and 2 on primary human monocytes is increased following IL-13/IL-4 treatment as is chemotaxis to CXCL8 [39]. These findings indicate that CXC chemokines may play a prominent role in allergic inflammation through interaction with IL-13 and IL-4. Our results are also in agreement with those reports demonstrating that airway hyperresponsiveness was not related to plasma IgE levels [40,41].

The data demonstrating that blockade of the CXC chemokines results in decreased circulating levels of IgE is both intriguing and novel. There are multiple pathways that drive IgE production, including the actions of IL-4 and IL-13 [42]. Currently, there is excitement about the use of therapies directed against IgE antibodies for the treatment of asthma [43]. There is virtually nothing known about chemokine regulation of IgE, although a recent report indicated that patients with hyper-IgE have reduced levels of the CXC chemokine ENA-78 [44]. The CXC chemokine regulation of IgE deserves further exploration.

Chemokine redundancy may account for contrasting results between our studies focusing on neutralization of ligands and those of De Sanctis et al. where the receptor is deficient [36]. Redundancy in the system is plausible considering that we have shown neutralization of either chemokine alone did not decrease pulmonary inflammation. This may also account for the continued neutrophil influx still observed following only KC or MIP-2 neutralization. The promiscuity of chemotactic responses is two-fold in that many ligands can bind to multiple receptors and most receptors bind to more than one ligand. Whether multiple chemokines binding to a single receptor is a matter of redundancy or differential regulation remains to be proven definitively. Our studies fit with those of Knott et al. which showed that administration of either anti-KC or anti-MIP-2 alone failed to effect pulmonary neutrophil influx in a murine model of OVA-induced asthma while anti-KC and anti-MIP-2 administered i.n. prior to OVA challenge led to a 69% decrease in BAL neutrophil levels [7]. As mentioned earlier CXCR2 is bound by chemokines in addition to KC and MIP-2 which could explain diverging results.

Asthma has long been considered a disease of the airways mediated by the Th2 cytokines IL-4, IL-5 and IL-13. The present study establishes that the mouse homologs of CXCL8, KC and MIP-2 modulate pulmonary inflammation and IgE secretion in a house dust extract-induced model of asthma and further dissociates pulmonary inflammatory responses from the development of airway hyperreactivity.

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