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

Laura McKinley, Jiyoun Kim, Gerald L. Bolgos, Javed Siddiqui, Daniel G. Remick*

Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109-0602, USA

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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.

Keywords: Allergy; Chemokines; Lipopolysaccharide; Neutrophils

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.

* Corresponding author. M2210, Medical Science Building I, 1301 Catherine Road, Ann Arbor, MI 48109-0602, USA. Tel.: +1 734 763 6454; fax: +1 734 763 6476.

E-mail address: remickd@umich.edu (D.G. Remick).

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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-m^2 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 antichemokine 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.