The Journal of Immunology

Eotaxin Represents the Principal Eosinophil Chemoattractant in a Novel Murine Asthma Model Induced by House Dust Containing Cockroach Allergens

Jiyoun Kim, Andrew C. Merry, Jean A. Nemzek, Gerry L. Bolgos, Javed Siddiqui and Daniel G. Remick

J Immunol 2001; 167:2808-2815; ;
doi: 10.4049/jimmunol.167.5.2808
http://www.jimmunol.org/content/167/5/2808

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Eotaxin Represents the Principal Eosinophil Chemoattractant in a Novel Murine Asthma Model Induced by House Dust Containing Cockroach Allergens[1]

Jiyoun Kim, Andrew C. Merry, Jean A. Nemzek, Gerry L. Bolgos, Javed Siddiqui, and Daniel G. Remick[2]

Asthma represents a serious health problem particularly for inner city children, and recent studies have identified that cockroach allergens trigger many of these asthmatic attacks. This study tested the concept that asthma-like pulmonary inflammation may be induced by house dust containing cockroach allergens. An aqueous extract was prepared from a house dust sample containing endotoxin and high levels of cockroach allergens. BALB/c mice were immunized with the house dust extract (HDE) and received two additional pulmonary challenges. Bronchoalveolar lavage (BAL) eosinophil counts and eotaxin levels were significantly increased in immunized mice exposed to the HDE, whereas neutrophils were the predominant BAL inflammatory cell in the unimmunized mice. Kinetics studies in immunized mice demonstrated a peak pulmonary inflammatory response 48 h after the last challenge. The allergic response in this model was further confirmed by histological and physiological studies demonstrating a significant influx of eosinophils and lymphocytes in the peribronchial area, and severe airway hyperreactivity through whole-body plethysmography. The specificity of the response was established by immunizing with HDE and challenging with purified cockroach allergen, which induced pulmonary eosinophilia and airway hyperreactivity. Ab inhibition of eotaxin significantly inhibited the number of BAL eosinophils. These data describe a novel murine model of asthma-like pulmonary inflammation induced by house dust containing endotoxin and cockroach allergens and further demonstrate that eotaxin represents the principal chemoattractant for the recruitment of the pulmonary eosinophils. ***The Journal of Immunology*****, 2001, 167: 2808–2815.**

Asthma has become a major public health issue in urban communities. In particular, low-income and minority children have the highest morbidity and mortality rates for asthma in the United States (1). A number of studies have shown that environmental allergen exposure, particularly that of the cockroach, is the major contributing factor for asthma exacerbations among inner-city children (2–4).

Asthma represents a unique form of chronic airway inflammation characterized by reversible airway obstruction and airway hyperreactivity (AHR)[3] (5). Upon exposure to the allergen, inflammatory cells including lymphocytes, macrophages, neutrophils, and eosinophils infiltrate the airway. Among these, eosinophils are predominant effector cells for tissue damage and pulmonary dysfunction (5, 6). The severity of AHR strongly correlates with the intensity of pulmonary infiltration by eosinophils (7–9).

When infiltrated into the lung after allergen challenge, eosinophils induce various inflammatory changes in the airways via release of a wide variety of immunomodulator molecules (6, 10) including major basic protein (11). The localization of eosinophils to the bronchial mucosa potentially primes the subsequent immune responses and augments allergic pulmonary inflammation by the secretion of various cytokines (5, 12). The critical role of the eosinophil in the immunopathology of asthma has been proven in clinical observations and animal models (6).

Selective recruitment of eosinophils into the airways during allergic inflammation suggests that eosinophil-specific chemoattractants are produced and released throughout the course of pulmonary inflammation. Since first identified and sequenced in a guinea pig model (13), the C-C chemokine eotaxin has been considered the major eosinophil chemoattractant in various animal models of eosinophilic pulmonary inflammation (14, 15) and in human tissues after allergen sensitization (16–18).

For the last 10 years, mouse models of asthma have been developed to study the inflammatory mechanisms of asthma (19). In an effort to develop allergic asthma-like pulmonary inflammation in healthy animals, mice were sensitized and/or challenged with various kinds of allergens. Among them, OVA (20) and purified indoor allergens such as cockroach (21) and dust mite (22) are commonly used allergens for murine models. However, the allergens used for these animal models may not represent the same constituents to which asthmatics are exposed throughout their daily life in terms of quality and quantity of allergens. To date, no murine model of asthma-like pulmonary inflammation has been developed by using allergens derived directly from house dust. Although allergens represent an important component of asthma, other initiating factors are certainly present in the environment.

Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109

Received for publication April 25, 2001. Accepted for publication July 9, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported in part by National Institute of Environmental Health Sciences Grant ES 09589 and U.S. Environmental Protection Agency Grant R826710-01.

[2] Address correspondence and reprint requests to Dr. Daniel G. Remick, M2210 Med Sci I, 1301 Catherine Road, Ann Arbor, MI 48109-0602. E-mail address: remickd@umich.edu

[3] Abbreviations used in this paper: AHR, airway hyperreactivity; BAL, bronchoalveolar lavage; HDE, house dust extract; DM, dilution medium; Mch, acetyl β-methacholine; WBP, whole-body plethysmography; Penh, enhanced pause; PMN, polymorphonuclear cell.

0022-1767/01/$02.00

Endotoxin is a component from the cell wall of Gram-negative bacteria, and its role in the pathogenesis of asthma has been suggested but is not well defined.

In an effort to establish a novel murine asthma model, we collected house dust from an inner-city house in Detroit, Michigan and used a simple aqueous extract for sensitization and intratracheal challenge. We investigated AHR, pulmonary cellular infiltration, and chemokine levels in response to the house dust extract (HDE). We also examined the role of the eotaxin in this model by neutralizing eotaxin with a specific anti-chemokine Ab. This model demonstrates the critical role for eotaxin in the recruitment of eosinophils during asthma-like pulmonary inflammation.

Materials and Methods

Animals

Female BALB/c mice (18–20 g) were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and maintained under standard laboratory conditions. The mice were housed in a temperature-controlled room with a 12-h dark/light cycle and allowed food and water ad libitum. The experiments described below were performed in accordance with the National Institutes of Health guidelines, and approval was obtained from the University of Michigan Animal Use Committee.

Household dust collection and extraction

Household dust was collected from 10 different houses in Detroit, Michigan. The dust samples were collected from a 1-m^2 area by using an electric vacuum cleaner with a dust collector (Indoor Biotechnologies, Charlottesville, VA). Sterile PBS (2 ml) was added to the dust, which was then mixed end-over-end on an orbital rotator (Roto-Shake Genie; Scientific Industries, Bohemia, NY) at 4°C for 16 h. The resuspended dust was centrifuged for 10 min at 4°C, 1000 × *g*, and the supernatant was collected for allergen measurement by an ELISA.

The household dust sample used for all sensitizations and airway challenges was collected from a house that showed a high level of the cockroach allergens *Blattella germanica* 1 (Bla g1) and 2 (Bla g2). A total 4.3 g of dust was collected and resuspended with 30 ml of sterile PBS for extraction as described above. After extraction, the supernatants were aliquoted and stored at −20°C until use.

Quantification of allergens by ELISA

Assays for six different indoor allergens including two house cockroach allergens (Bla g1 and Bla g2), two house mite allergens (*Dermatophagoides pteronyssinus*, Der p1 and *Dermatophagoides farinae*, Der f1), one cat allergen (*Felis demesticus*, Fel d1), and one dog allergen (*Canis familiaris*, Can f1) were performed by ELISA. All Abs and standards for these ELISAs were purchased from Indoor Biotechnologies. For Bla g1, Bla g2, and Can f1, 96-well plates (Nunc Immunoplate Maxisorb; Nunc, Neptune, NJ) were coated with anti-Bla g1 mAb, anti-Bla g2 mAb, or anti-Can f1 mAb, respectively, and incubated overnight at room temperature. The plates were then washed using a wash buffer containing 0.05% Tween 20 (FisherBiotech, Fair Lawn, NJ) in PBS. Nonspecific binding sites were blocked by incubating the plates with Blocker Casein (Pierce, Rockford, IL) in PBS for 1 h. This and subsequent incubations were conducted at room temperature on a shaker. After washing, samples were added and the plates incubated for 1 h. Standard curves were prepared using the appropriate recombinant allergen. All standard and sample dilutions were made in dilution medium (DM; 10% casein in PBS supplemented with 0.01% normal rabbit plasma and 0.05% Tween 20 (FisherBiotech)) for nonspecific blocking. Plates were washed and rabbit polyclonal anti-cockroach antiserum (diluted in DM) for Bla g1 and Bla g2, and rabbit polyclonal anti-Can f1 antiserum for Can f1, were used as detection Abs. Plates were incubated for 1 h. After washing, HRP-conjugated goat anti-rabbit IgG (BioSource International, Camarillo, CA) was added and plates were incubated for 1 h. After a final wash, 3,3′,5,5′ tetramethylbenzidine (Genzyme Diagnostics, San Carlos, CA) was added, plates were incubated in the dark for 15 min, and the reaction was stopped with 1.5 N H_2SO_4. Plates were read using dual wavelengths (465 and 590 nm) on the Bio-Tek microplate reader (Bio-Tek Instruments, Winooski, VT), and allergen concentrations were estimated using the recombinant allergen standard curve.

For Der p1, Der f1, and Fel d1, 96-well plates were coated with anti-Der p1, anti-Der f1, or anti-Fel d1 mAb, respectively, and incubated overnight at room temperature. The plates were then washed and processed as above. Biotinylated anti-Der p1 Ab (diluted in DM), biotinylated anti-Der f1, or biotinylated anti-Fel d1 mAb was used as detection Ab followed by HRP-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA) and 3,3′,5,5′ tetramethylbenzidine for color development. The lower limits of detection were 0.008 U/ml for Bla g1, 0.31 ng/ml for Bla g2, 3.13 ng/ml for Der p1, 0.39 ng/ml for Der f1, 0.03 mU/ml for Fel d1, and 3.13 IU/ml for Can f1.

Development of murine model of asthma-like pulmonary inflammation

For the development of the model, mice were sensitized by an i.p. injection of the HDE mixed with an adjuvant and then given two separate pulmonary challenges. Specifically, on day 0, mice were sensitized by i.p. injection of 50 μl of HDE emulsified in 50 μl TiterMax Gold (CytRx, Norcross, GA) for a total volume of 100 μl. TiterMax has been shown to induce a strong immune response with minimal tissue swelling (23). On days 14 and 21, mice were given an airway challenge of 50 μl of HDE while under anesthesia with methoxyflurane (Metofane; Shering-Plough, Union, NJ) (24). Briefly, an anesthetized mouse was suspended on its back on an inclined board by its teeth. The body weight was supported by taping the base of the tail to the board. While under anesthesia the jaw was opened and the tongue gently extended with forceps. This positions the epiglottis with the trachea open. An aliquot of the HDE (50 μl) was placed at the base of the oropharynx, which temporarily occludes air flow and is subsequently inhaled. The technique produces a reliable delivery of fluid droplets to the lung. The unimmunized group did not receive the i.p. injection on day 0 but received two airway challenges on days 14 and 21. For the specificity of the model, mice were also challenged with the house dust and received one airway challenge on day 7. For the second airway challenge the mice received either purified cockroach allergen, Bla g2 or purified dust mite allergen, Der p1. Forty-eight hours from last airway challenge, AHR to acetyl β-methacholine (Mch) was measured, and the mice were sacrificed for histologic examination and collection of bronchoalveolar lavage (BAL).

AHR

AHR of mice to increasing doses of aerosolized Mch (Sigma, St. Louis, MO) was measured in unrestrained and conscious animals by a whole-body plethysmography (WBP) system (Buxco, Troy, NY). After the box was calibrated, the pressure difference between the main chamber of the WBP containing the mouse and a reference chamber was measured. The difference of the box signal results from changes in volume and resultant pressure in the main chamber during each respiratory cycle of the animal. Inspiration and expiration were processed as a waveform of the box pressure-time signal and recorded by a computer data-acquisition system. Changes in early expiration during bronchoconstriction will alter the waveform of the box pressure-time and can be quantified. These quantified alterations are reflected by the main indicator of airway obstruction, enhanced pause (Penh). Penh is strongly correlated with airway resistance of the animal and is widely used in murine asthma models (25).

After mice were acclimated to the main chamber, baseline Penh was measured for 5 min. Either aerosolized PBS or Mch (Sigma) in increasing concentrations (6, 12, 25, and 50 mg/ml) was nebulized through the inlet of the main chamber for 2 min, and response to each dose was measured for 5 min. The average Penh for 5 min was used to compare the results among experimental groups. Airway responsiveness was expressed as a percent increase of Penh for each dose of Mch compared with Penh for PBS challenge.

Peripheral blood analyses

For blood counting, 20 μl of EDTA (Sigma) anti-coagulated blood was collected from the tail as previously described (26) at 48 h after the second airway challenge. A Hemavet Mascot Multispecies Hematology System Counter 1500R (CDC Technologies, Oxford, CT) was then used to perform a complete blood count. At the time of sacrifice, blood was also collected from the retro-orbital venous plexus into tubes containing 50 U of porcine derived heparin (Elkins-Sinn, Cherry Hill, NJ), and plasma was stored at −20°C.

BAL analyses

After blood collection, mice were euthanized by cervical dislocation. For the BAL, the trachea was exposed and then intubated with a polyethylene catheter. BAL fluid was harvested by lavaging with two separate aliquots of 1 ml of HBSS (Life Technologies, Grand Island, NY) through the trachea. The first wash was centrifuged, and the BAL supernatant was stored as above. The second wash was centrifuged, the

Table I. *Total allergen levels in HDE*[a]

	Cockroach		Dust Mite		Cat	Dog
	Bla g1 (U/ml)	Bla g2 (ng/ml)	Der p1 (ng/ml)	Der p2 (ng/ml)	Fel d1 (mU/ml)	Can f1 (IU/ml)
House no. 5	377	6249	<3.1	<1.6	44	23

[a] A total of 4.3 g of house dust was extracted into 30 ml of sterile PBS. All allergen levels were determined by ELISA. Bla g1 and Bla g2, German cockroach (*B. germanica*) g1 and g2; Der p1, house dust mite (*D. pteronyssinus*; Der f1, Der f1, house dust mite (*D. farinae*); Fel d1, cat (*F. domesticus*); Can f1, dog (*C. familiaris*).

cell pellet from the first wash was pooled with the cell pellet from the second, and a total cell count was obtained using a Coulter counter model ZF (Coulter Electronics, Hialeah, FL). Cytospin slides were prepared and stained with Diff-Quick (Baxter, Detroit, MI), and differentials were obtained after counting 300 cells.

Lung histology

The left lung lobe and trachea were removed, fixed in formalin, and processed for routine histology in paraffin.

Mediator measurement

Eotaxin was measured using matched Ab pairs via ELISA (R&D Systems, Minneapolis, MN) using our previously described methods (26). Endotoxin was measured with a chromogenic *Limulus* assay following the manufacturer's instructions (BioWhittaker, Walkersville, MD).

Anti-eotaxin treatment

Groups of normal BALB/c mice were sensitized and challenged with the HDE as described above. Twenty-four hours after the second challenge (day 22), mice were given 10 μg of rat anti-murine eotaxin mAb (R&D Systems) or 10 μg of control rat IgG (Jackson ImmunoResearch Laboratories) by intratracheal challenge. Forty-eight hours after the last airway challenge, AHR was measured and the cellular constituents in the BAL fluid were determined.

Statistical analyses

Summary statistics were expressed as mean ± SEM in all figures. Differences between all treatment groups were compared by ANOVA. A Tukey test for pair-wise comparisons was performed when the overall F value was statistically significant (p 0.05). Eotaxin levels that were not detectable were assigned a value equal to half the lower limit of detection for that assay.

Results

Screening house dust for cockroach allergen

Cockroach infestation and sensitization to these allergens are strongly correlated with the development of allergic respiratory disease, especially asthma (3). A major objective of the present study was to establish a novel murine model of asthma using house dust containing high levels of endogenous cockroach allergens instead of immunizing the mice with purified or recombinant allergens. In an effort to locate a house containing dust with a high level of cockroach allergen, house dust was collected from 10 houses in Detroit, Michigan. The dust samples were collected from a 1-m^2 area in the kitchen by using a dust collector attached to an electric vacuum cleaner. After being transported to our laboratory, each house dust sample was extracted with sterile PBS and screened for six different indoor allergens; German cockroach (*B. germanica*, Bla g1 and Bla g2), house dust mite (*D. pteronyssinus*, Der p1 and *D. farinae*, Der f1), cat (*F. domesticus*, Fel d1), and dog (*C. familiarizs*, Can f1). Based on this screening, we selected the house that contained the highest concentration of cockroach allergen, and a large quantity of house dust was collected extensively from the house. A total of 4.3 g of house dust was collected and extracted with 30 ml sterile PBS.

We assayed for six different indoor allergens in this extract by using an ELISA (Table I). Our HDE contained high concentrations of cockroach allergen (378 U/ml Bla g1 and 6249 ng/ml Bla g2), whereas Der p1, Der f1, Fel d1, and Can f1 allergen levels were very low. In addition to quantification of various allergens, the endotoxin level of the HDE was measured by a *Limulus* assay, which detected 270 pg/ml endotoxin in this extract. This aqueous HDE was used for immunization and intratracheal instillation for all experiments throughout this study. Initial dose-response studies showed significant increases in pulmonary inflammation from the mice immunized and challenged with 1/10 diluted extract as well as with undiluted extract (data not shown). This dilution (1/10) was used for all subsequent studies.

Pulmonary inflammation after HDE sensitization and intratracheal instillation

An increase in the number of eosinophils in the airways has been considered a hallmark sign of allergic asthma (5, 27). To characterize pulmonary inflammation in this mouse model, BALB/c mice were sensitized and intratracheally challenged with the HDE containing high concentrations of cockroach allergens. The number of eosinophils and neutrophils in the BAL was quantitated at 12-h intervals after the last challenge. The eosinophil counts progressively increased in the lung lavage from this model (Fig. 1). Eosinophil infiltration started within 12 h after the last intratracheal challenge and reached maximum levels at 48 h (Fig. 1). After 48 h, the number of eosinophils dropped but remained elevated compared with the 12-h time

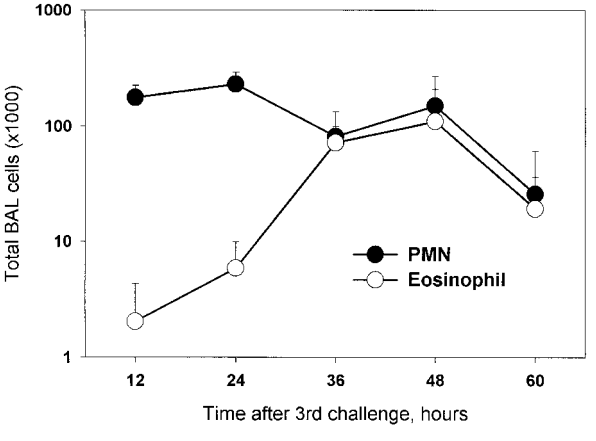

FIGURE 1. Kinetics of pulmonary inflammatory cell recruitment in mouse lung lavage after HDE challenge. Each group of mice was immunized on day 0 and intratracheally challenged with diluted HDE on days 14 and 21. Groups of mice were sacrificed every 12 h after the last challenge. BAL fluid was harvested through the trachea, the total cells were counted, and differential counts were performed on stained cytospin preparations. Values represent mean ± SEM with n = 3–5 for each group. Note the log scale for the number of cells.

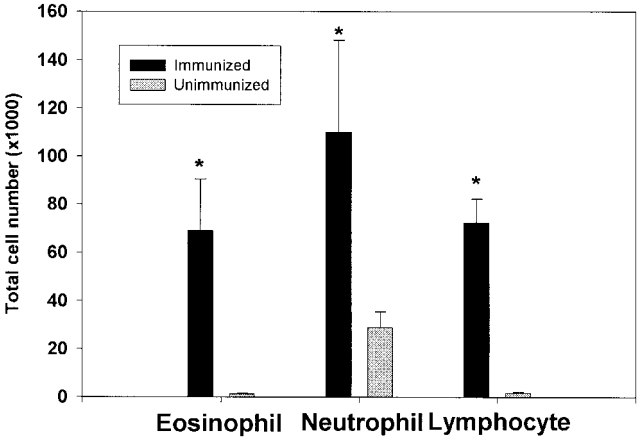

FIGURE 2. Eosinophil, PMN, and lymphocyte recruitment in mouse lung. All leukocyte counts were performed 48 h after last intratracheal challenge. Values represent mean ± SEM with $n = 8–16$ for each group. *, $p < 0.05$ when compared with unimmunized mice.

point. Based on this observation, animals were sacrificed 48 h after the last intratracheal administration of the allergen in subsequent studies. Interestingly, a significant number of polymorphonuclear cells (PMNs) in the lung lavage were observed at the early time point (12 h). Early recruitment of PMNs is probably due to the endotoxin present in the HDE. To further characterize the model, immunized and unimmunized mice were compared. Eosinophils and PMNs accumulated in the BAL were measured 48 h after the last pulmonary challenge (Fig. 2). The number of eosinophils in immunized mice was significantly higher than in the unimmunized group, indicating that prior sensitization was required for the development of the asthma-like inflammatory response. Additionally, there were significantly more lymphocytes recovered from the BAL fluid of the immunized mice (Fig. 2).

Eosinophil recruitment in the lung of house dust-challenged mice was further confirmed by histological studies (Fig. 3). Mice were either unimmunized or immunized according to the protocol. Lungs were processed for histology 48 h after the last challenge, and after BAL was performed. In the unimmunized mice, the pulmonary histology was essentially normal (Fig. 3*A*). In contrast, the immunized mice had a significant influx of inflammatory cells including lymphocytes and eosinophils. These cells were located in the peribronchial space as well as in the perivascular space (Fig. 3*B*). Higher power magnification demonstrated that the cells were both eosinophils and lymphocytes (Fig. 3*C*). It is important to note that the histology was prepared after the lungs were lavaged, which accounts for the lack of eosinophils within the airways.

To confirm whether pulmonary inflammation was a localized reaction rather than a part of systemic inflammation, peripheral blood was collected and a complete blood count performed 48 h after the second airway challenge. There were no significant differences in circulating eosinophil numbers between normal, immunized, and unimmunized mice (Table II).

Airway hyperresponsiveness after HDE challenge

The number of eosinophils infiltrated into the lung is closely correlated with AHR in many mouse models of asthma (5, 8). Penh from WBP represents an accepted measure of AHR in unrestrained and conscious animals, and Penh changes are closely related to the pulmonary recruitment of eosinophils in asthmatic animals (25). We measured the responses to inhaled Mch in immunized and unimmunized animals (48 h after last intratracheal challenge) to investigate how pulmonary function is affected by house dust containing high concentrations of cockroach allergens. The control group (unimmunized but intratracheally challenged) showed no significant changes in Penh in response to increasing doses of aerosolized Mch (Fig. 4). In contrast, the Penh of the immunized group was substantially increased with escalating doses (25 and 50 mg/ml) of aerosolized Mch compared with control mice.

We next investigated the specificity of pulmonary inflammatory reaction in this mouse model by using purified or recombinant allergens. Mice were immunized and challenged once with the HDE. For the second challenge, the same quantity of purified cockroach allergen, Bla g2 (31 ng) found in the HDE was used. For a control, recombinant house dust mite allergen, Der p1, (31 ng) was used for the second challenge. We used an irrelevant Ag for the control rather than only using normal saline to further define that the response was specific to the cockroach allergen. The Bla g2-challenged mice showed significantly more eosinophils in lung lavage than Der p1-challenged mice (Fig. 5*A*). These mice also had enhanced AHR with substantially higher Penh values (Fig. 5*B*).

Role of eotaxin in allergic airway inflammation induced by house dust containing cockroach allergens

Selective recruitment of eosinophils into the lungs of immunized mice suggests the presence of eosinophil-specific chemoattractants in the lung during pulmonary inflammation. Eotaxin, a CC chemokine, is the hallmark eosinophil chemoattractant released in the

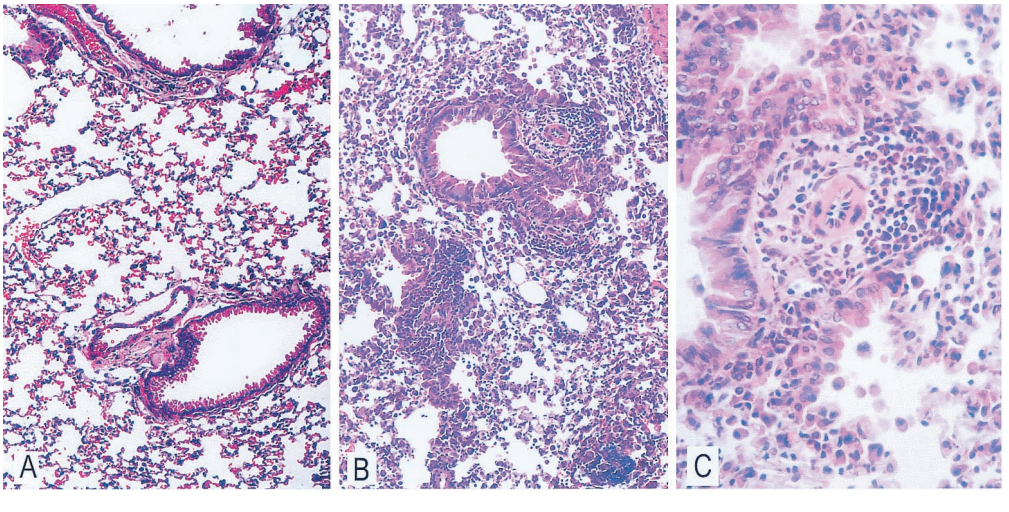

FIGURE 3. Histology of lung tissue after challenge with HDE in immunized and unimmunized mice. Localization of eosinophils and lymphocytes is shown. Lungs were harvested 48 h after the last pulmonary challenge (and after BAL was performed). *A*, Representative histology from an unimmunized mouse with essentially normal histology. *B*, Histology from an immunized mouse with a marked peribronchial infiltrate of eosinophils and lymphocytes. *C*, High power magnification of an area in *B* demonstrating the eosinophils.

Table II. *Peripheral blood eosinophils*[a]

	Immunized	Unimmunized	Normal
Eosinophils ($\times 10^6$/ml)	0.160 ± 0.049	0.100 ± 0.009	0.130 ± 0.013

[a] Group of BALB/c mice was immunized or nonimmunized as described in *Materials and Methods*, and the peripheral blood eosinophils were determined 48 h after the last challenge. Values represent mean ± SEM with $n = 5$ for each group.

lung in many animal models of eosinophilic airway inflammation (13, 28). We measured eotaxin levels in the lung lavage to investigate the involvement of this chemokine in eosinophil accumulation in the lung using the HDE-immunized mouse model (Fig. 6). BAL eotaxin levels in the immunized mice were significantly higher than in the unimmunized mice.

The role of eotaxin in this asthmatic pulmonary inflammation was evaluated by administration of neutralizing Ab to the immunized mice. The immunized mice were treated with either 10 μg of rat anti-mouse-eotaxin mAb or 10 μg of rat IgG. The role of eotaxin in the pulmonary inflammation was evaluated by quantitating the number of eosinophils in the lung lavage (Fig. 7). The number of eosinophils in the BAL of immunized and anti-eotaxin Ab-treated animals was substantially lower than immunized and control Ab-treated mice. This provides strong evidence that eotaxin represents a critical mediator of asthmatic pulmonary inflammation induced by HDE containing a high concentration of house cockroach allergens.

Discussion

Asthma is one of the most common illnesses in industrialized countries and affects 8–10% of children and 3–5% of the adult population. In the United States, ~15 million Americans suffer from asthma (19, 29). The morbidity and mortality due to asthma have increased for the last 30 years in the Western world; the reasons for this recent increase have not been fully defined (29). Several explanations for the increase in asthma have been proposed such as elevated levels of potential sensitizing agents, i.e., tobacco smoke and other organic chemicals, and diminishing bacterial infections that generally promote differentiation of a Th1 immune response (29). Increased levels of indoor allergens including dust mite, cockroach, and pet dander represent other possible reasons. Recent published studies have demonstrated that cockroach allergen sensitization correlates with the morbidity due to asthma because in some inner-city areas nearly 37% of asthmatic children (3) and 48% of asthmatic adults (30) are allergic to cockroach allergens. Thus, it is of great interest to develop experimental murine models that reproducibly demonstrate the major characteristics of asthma by using house dust.

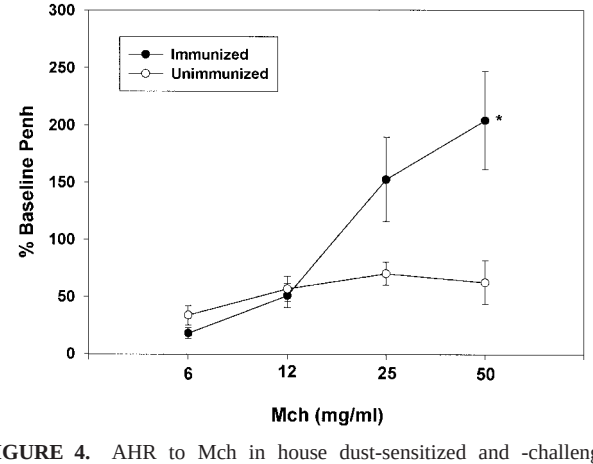

FIGURE 4. AHR to Mch in house dust-sensitized and -challenged BALB/c mice. Penh values were obtained in response to increasing concentrations of nebulized Mch. The data are expressed as the mean ± SEM of Penh values as the percentage of baseline observed after PBS nebulization. $n = 4$–5 for each group. *, $p < 0.05$ when compared with unimmunized mice.

Reversible airway obstruction in response to allergens, chronic airway eosinophilia, and AHR are characteristics of asthma (31). Among these, chronic eosinophilic pulmonary inflammation represents an obvious hallmark of allergic asthma (32). We have examined the asthma-like inflammatory changes in mice immunized and intratracheally challenged with house dust containing high concentrations of cockroach allergens and have investigated the role of eotaxin in the pulmonary recruitment of eosinophils in this model.

In this study, eosinophil infiltration was initiated within 12 h after the second intratracheal challenge of house dust and reached a peak at 48 h. The number of eosinophils recruited in the BAL of the immunized mice was significantly higher than that of unimmunized mice. Pulmonary eosinophilia was further confirmed by lung histology. Compared with unimmunized mice, substantial numbers of leukocytes are recruited in immunized mice and most of the infiltrated cells are either eosinophils or lymphocytes. The early rise in the number of neutrophils in BAL fluid is probably due to the endotoxin contamination in our HDE. These results were not surprising because the HDE contained high concentrations of endotoxin (270 pg/ml). We collected the house dust for these studies from the kitchen, where the cockroach allergen level is higher than other areas (33) and presumably there is a greater possibility of bacterial growth in the dust collected from that area. Previous studies have also shown that neutrophils are the first cell type recruited into the lung in response to allergen challenge (27, 34). Our results are similar to other studies regarding the kinetics of eosinophil recruitment where pulmonary eosinophilia peaked at 48 h after the last allergen challenge in cockroach allergen-sensitized mouse model (35). Pulmonary eosinophilia peaked 3 h after the last challenge in an OVA-sensitized and -challenged mouse model (15) and peaked in 24 h after the last challenge in a cockroach whole body extract-sensitized and -challenged guinea pig model (21).

Extensive studies have demonstrated that BAL fluid of patients with bronchial asthma showed increased number of eosinophils (36, 37), which correlates with AHR (5) and disease severity (38). In the present study, AHR to aerosolized Mch was induced in house dust-sensitized and intratracheally challenged mice as measured through WBP, whereas no enhanced AHR was observed in unimmunized mice. Therefore, the pulmonary recruitment of eosinophils in this mouse model paralleled the development of airflow obstruction. Thus, it is concluded that the characteristics of bronchial asthma, including eosinophilic pulmonary inflammation and AHR, were induced in mice sensitized and challenged with house dust containing a high concentration of cockroach allergens.

The role of the cockroach allergens in this model was further confirmed by Ag-specific induction of pulmonary eosinophilia and airway obstruction. In these experiments, mice from each group were treated identically except for the second intratracheal challenge, in which mice were given the same amount of either Bla g2 or Der p1

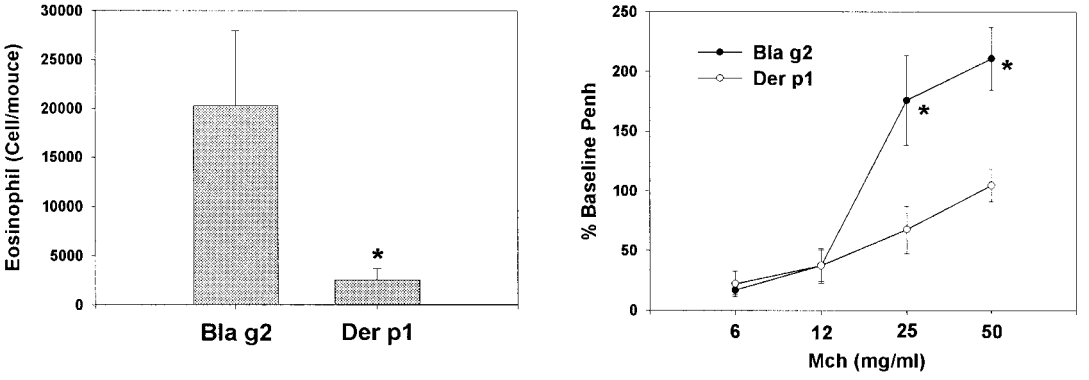

FIGURE 5. Eosinophil counts in lung lavage (*A*) and AHR (*B*) from purified allergen-treated mice. Each group of mice was sensitized with house dust and challenged identically except for the second challenge. Corresponding allergen (31 ng of Bla g2 or Der p1) was administered by intratracheal challenge on day 21. All leukocytes were harvested 48 h after last intratracheal challenge. AHR was measured immediately before harvesting BAL. Values represent mean ± SEM with $n = 6–8$ for each group. ∗, $p < 0.05$ when compared with Bla g2-treated mice.

allergen. The number of eosinophils recruited in the lung and the Penh of Bla g2-treated mice were significantly higher than those of Der p1-treated mice and substantially similar to those of house dust-treated mice. Thus, it is suggested that cockroach allergens in the HDE play the major role in pulmonary eosinophilia and AHR observed in this mouse model of asthma. The allergen-specific pulmonary infiltration of eosinophils in this model is very similar to the asthmatic responses demonstrated in the aerosolized whole-body extract of cockroach-sensitized guinea pig models (21) and in a mouse model using purified cockroach allergen (35). The cockroach-sensitized guinea pigs showed a dose-response relationship between the number of eosinophils recruited in the BAL and the amount of cockroach allergen. In the mouse model, very similar kinetics of cockroach allergen-specific induction of pulmonary eosinophilia and AHR were demonstrated as shown in our model.

Eotaxin represents one of the most efficient eosinophil chemoattractants and plays a key role in allergic airway inflammation. In previous studies, the levels of eotaxin expressed correlated with pulmonary eosinophilia (5, 15, 39). In murine models, neutralization of eotaxin significantly inhibited eosinophilic inflammation and AHR following allergen challenge (40). In our study, significant amounts of eotaxin were expressed in immunized mice, and the level of eotaxin in BAL showed a dose-response relationship with the amount of allergen used for sensitization (data not shown). Furthermore, the number of eosinophils accumulated in BAL fluid was substantially reduced by neutralization of eotaxin with anti-eotaxin Ab. These results indicate that eotaxin is responsible for the pulmonary infiltration of eosinophils in response to cockroach allergen challenge.

The role of LPS (or endotoxin) in the pathogenesis of asthma has become a recent subject of investigation. It has been postulated that LPS exposure during early life will induce Th1 cytokines such as IFN-γ and IL-12. The cytokines will diminish the synthesis of Th2 cytokines including IL-4, -5, and -13 (41). Based on this hypothesis, LPS exposure during the priming phase of an allergic response would result in decreased asthma. Recent clinical results have shown that children with high levels of endotoxin in the house dust have less allergic sensitization (41). However, conflicting results are found when evaluating adults. In these studies the severity of asthma was positively correlated with the levels of endotoxin within the house dust, with worse asthma associated with higher levels of endotoxin (42). Additionally, increased numbers of eosinophils were found after low-level endotoxin challenge in humans (43). In a murine model of asthma induced by OVA, LPS exposure results in increased numbers of inflammatory cells

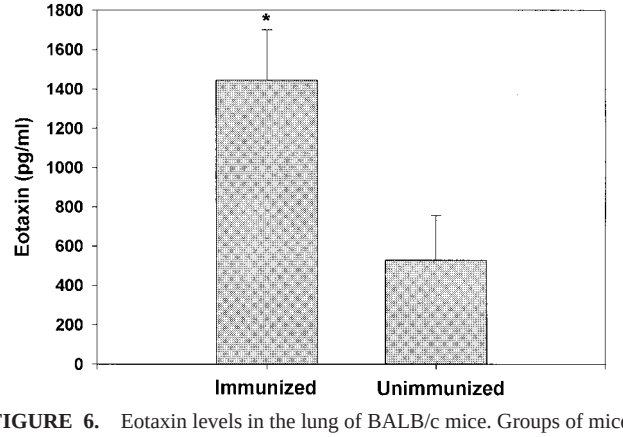

FIGURE 6. Eotaxin levels in the lung of BALB/c mice. Groups of mice were sensitized and challenged with the HDE. Chemokine levels were determined by ELISA in the lung lavage from immunized and unimmunized mice harvested at 48 h after the last challenge. Values represent mean ± SEM with $n = 6–9$ for each group. ∗, $p < 0.05$ when compared with unimmunized mice.

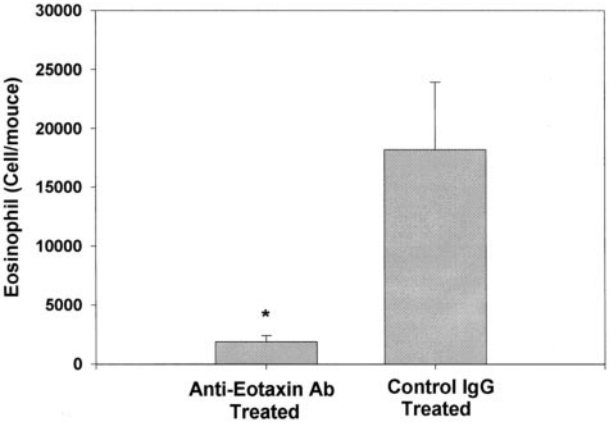

FIGURE 7. Eosinophil counts in lung lavage from anti-eotaxin Ab or control rat IgG-treated mice. Each group of mice was sensitized and challenged with the HDE. The Ab (10 μg) was administered by intratracheal challenge 24 h after last challenge and BAL leukocytes were harvested 48 h after last intratracheal challenge. Values represent mean ± SEM with $n = 5$ for each group. ∗, $p < 0.05$ when compared with control IgG-treated mice.

within the lung (44). Therefore, the relationship between endotoxin exposure and the subsequent development of asthma is not clear. Another consideration in this model is the use of the aqueous extract of the house dust rather than the dust itself. It is possible that allergens collected by using a vacuum cleaner may not precisely represent the aeroallergens in the air, which are actually inhaled. The size and weight of some aeroallergens may prevent them from settling to the floor and/or from being trapped in the dust collection tube of the vacuum cleaner.

There are some important similarities and differences between this novel model of asthma and other models based on OVA or purified cockroach allergens. The similarities include the development of asthma-like pulmonary inflammation with typical histopathology, eosinophils in the airways, and AHR. Some important differences include the early recruitment of neutrophils and the fact that very low levels of allergen (31 ng) are required to trigger the pulmonary inflammation.

The data we presented here have demonstrated the characteristics of a mouse model of asthma in response to house dust that contains the environmental allergens found in the German cockroach as well as endotoxin. Cockroach allergen sensitization of mice elicits asthma-like pulmonary inflammation characterized by eosinophilia and AHR. The pulmonary eosinophil recruitment is dependent upon pulmonary expression of eotaxin. This novel murine model of asthma induced by house dust represents a valuable tool for further study and may permit the dissection of the allergen-endotoxin interactions important in the pathogenesis of asthma.

Acknowledgments

The Michigan Center for the Environment and Children's Health (MCECH) is a community-based participatory research initiative investigating the influence of environmental factors on childhood asthma. MCECH involves collaboration among the University of Michigan Schools of Public Health and Medicine, the Detroit Health Department, the Michigan Department of Agriculture, Plant and Pest Management Division, and nine community-based organizations in Detroit (Butzel Family Center, Community Health and Social Services Center, Detroiters Working for Environmental Justice, Detroit Hispanic Development Corporation, Friends of Parkside, Kettering/Butzel Health Initiative, Latino Family Services, United Community Housing Coalition and Warren/Conner Development Coalition), and Henry Ford Health System.

References

1. Krieger, J. W., L. Song, T. K. Takaro, and J. Stout. 2000. Asthma and the home environment of low-income urban children: preliminary findings from the Seattle-King County healthy homes project. *J. Urban Health 77:50.*
2. Kang, B. 1976. Study on cockroach antigen as a probable causative agent in bronchial asthma. *J. Allergy Clin. Immunol. 58:357.*
3. Rosenstreich, D. L., P. Eggleston, M. Kattan, D. Baker, R. G. Slavin, P. Gergen, H. Mitchell, K. McNiff-Mortimer, H. Lynn, D. Ownby, and F. Malveaux. 1997. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N. Engl. J. Med. 336:1356.*
4. Eggleston, P. A., R. A. Wood, C. Rand, W. J. Nixon, P. H. Chen, and P. Lukk. 1999. Removal of cockroach allergen from inner-city homes. *J. Allergy Clin. Immunol. 104:842.*
5. Rankin, S. M., D. M. Conroy, and T. J. Williams. 2000. Eotaxin and eosinophil recruitment: implications for human disease. *Mol. Med. Today 6:20.*
6. Seminario, M. C., and G. J. Gleich. 1994. The role of eosinophils in the pathogenesis of asthma. *Curr. Opin. Immunol. 6:860.*
7. Ferguson, A. C., M. Whitelaw, and H. Brown. 1992. Correlation of bronchial eosinophil and mast cell activation with bronchial hyperresponsiveness in children with asthma. *J. Allergy Clin. Immunol. 90:609.*
8. Hogan, S. P., A. Mould, H. Kikutani, A. J. Ramsay, and P. S. Foster. 1997. Aeroallergen-induced eosinophilic inflammation, lung damage, and airway hyperreactivity in mice can occur independently of IL-4 and allergen-specific immunoglobulins. *J. Clin. Invest. 99:1329.*
9. Mattoli, S., M. A. Stacey, G. Sun, A. Bellini, and M. Marini. 1997. Eotaxin expression and eosinophilic inflammation in asthma. *Biochem. Biophys. Res. Commun. 236:299.*
10. Rothenberg, M. E. 1998. Eosinophilia. *N. Engl. J. Med. 338:1592.*
11. Gundel, R. H., L. G. Letts, and G. J. Gleich. 1991. Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J. Clin. Invest. 87:1470.*
12. Mould, A. W., A. J. Ramsay, K. I. Matthaei, I. G. Young, M. E. Rothenberg, and P. S. Foster. 2000. The effect of IL-5 and eotaxin expression in the lung on eosinophil trafficking and degranulation and the induction of bronchial hyperreactivity. *J. Immunol. 164:2142.*
13. Jose, P. J., D. A. Griffiths-Johnson, P. D. Collins, D. T. Walsh, R. Moqbel, N. F. Totty, O. Truong, J. J. Hsuan, and T. J. Williams. 1994. Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J. Exp. Med. 179:881.*
14. Rothenberg, M. E., A. D. Luster, and P. Leder. 1995. Murine eotaxin: an eosinophil chemoattractant inducible in endothelial cells and in interleukin 4-induced tumor suppression. *Proc. Natl. Acad. Sci. USA 92:8960.*
15. Gonzalo, J. A., C. M. Lloyd, L. Kremer, E. Finger, A. C. Martinez, M. H. Siegelman, M. Cybulsky, and J. C. Gutierrez-Ramos. 1996. Eosinophil recruitment to the lung in a murine model of allergic inflammation: the role of T cells, chemokines, and adhesion receptors. *J. Clin. Invest. 98:2332.*
16. Ponath, P. D., S. Qin, D. J. Ringler, I. Clark-Lewis, J. Wang, N. Kassam, H. Smith, X. Shi, J. A. Gonzalo, W. Newman, et al. 1996. Cloning of the human eosinophil chemoattractant, eotaxin: expression, receptor binding, and functional properties suggest a mechanism for the selective recruitment of eosinophils. *J. Clin. Invest. 97:604.*
17. Garcia-Zepeda, E. A., M. E. Rothenberg, R. T. Ownbey, J. Celestin, P. Leder, and A. D. Luster. 1996. Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia. *Nat. Med. 2:449.*
18. Pueringer, R. J., and G. W. Hunninghake. 1992. Inflammation and airway reactivity in asthma. *Am. J. Med. 92:32.S.*
19. Bice, D. E., J. Seagrave, and G. H. Y. Green. 2000. Animal model of asthma: potential usefulness for studying health effects of inhaled particles. *Inhal. Toxicol. 12:829.*
20. de Siqueira, A. L., M. Russo, A. A. Steil, S. Facincone, M. Mariano, and S. Jancar. 1997. A new murine model of pulmonary eosinophilic hypersensitivity: contribution to experimental asthma. *J. Allergy Clin. Immunol. 100:383.*
21. Zhou, D., G. Chen, J. T. Kim, L. Y. Lee, and B. C. Kang. 1998. A dose-response relationship between exposure to cockroach allergens and induction of sensitization in an experimental asthma in Hartley guinea pigs. *J. Allergy Clin. Immunol. 101:653.*
22. Clarke, A. H., W. R. Thomas, J. M. Rolland, C. Dow, and R. M. O'Brien. 1999. Murine allergic respiratory responses to the major house dust mite allergen Der p 1. *Int. Arch. Allergy Immunol. 120:126.*
23. Robuccio, J. A., J. W. Griffith, E. A. Chroscinski, P. J. Cross, T. E. Light, and C. M. Lang. 1995. Comparison of the effects of five adjuvants on the antibody response to influenza virus antigen in guinea pigs. *Lab. Anim. Sci. 45:420.*
24. Gavett, S. H., D. J. O'Hearn, X. Li, S. K. Huang, F. D. Finkelman, and M. Wills-Karp. 1995. Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J. Exp. Med. 182:1527.*
25. Hamelmann, E., J. Schwarze, K. Takeda, A. Oshiba, G. L. Larsen, C. G. Irvin, and E. W. Gelfand. 1997. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care Med. 156:766.*
26. Ebong, S. J., D. R. Call, G. Bolgos, D. E. Newcomb, J. I. Granger, M. O'Reilly, and D. G. Remick. 1999. Immunopathologic responses to non-lethal sepsis. *Shock 12:118.*
27. Sampson, A. P. 2000. The role of eosinophils and neutrophils in inflammation. *Clin. Exp. Allergy 30(Suppl. 1):22.*
28. Rothenberg, M. E., J. A. MacLean, E. Pearlman, A. D. Luster, and P. Leder. 1997. Targeted disruption of the chemokine eotaxin partially reduces antigen-induced tissue eosinophilia. *J. Exp. Med. 185:785.*
29. Eggleston, P. A., T. J. Buckley, P. N. Breysse, M. Wills-Karp, S. R. Kleeberger, and J. J. Jaakkola. 1999. The environment and asthma in U.S. inner cities. *Environ. Health Perspect. 107:439.*
30. Kang, B. C., J. Johnson, and C. Veres-Thorner. 1993. Atopic profile of inner-city asthma with a comparative analysis on the cockroach-sensitive and ragweed-sensitive subgroups. *J. Allergy Clin. Immunol. 92:802.*
31. Gleich, G. J. 1990. The eosinophil and bronchial asthma: current understanding. *J. Allergy Clin. Immunol. 85:422.*
32. Wills-Karp, M. 1999. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu. Rev. Immunol. 17:255.*
33. Gergen, P. J., K. M. Mortimer, P. A. Eggleston, D. Rosenstreich, H. Mitchell, D. Ownby, M. Kattan, D. Baker, E. C. Wright, R. Slavin, and F. Malveaux. 1999. Results of the National Cooperative Inner-City Asthma Study (NCICAS) environmental intervention to reduce cockroach allergen exposure in inner-city homes. *J. Allergy Clin. Immunol. 103:501.*
34. Smith, H. R., G. L. Larsen, R. M. Cherniack, S. E. Wenzel, N. F. Voelkel, J. Y. Westcott, and R. A. Bethel. 1992. Inflammatory cells and eicosanoid mediators in subjects with late asthmatic responses and increases in airway responsiveness. *J. Allergy Clin. Immunol. 89:1076.*
35. Campbell, E. M., S. L. Kunkel, R. M. Strieter, and N. W. Lukacs. 1998. Temporal role of chemokines in a murine model of cockroach allergen-induced airway hyperreactivity and eosinophilia. *J. Immunol. 161:7047.*
36. Beasley, R., W. R. Roche, J. A. Roberts, and S. T. Holgate. 1989. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am. Rev. Respir. Dis. 139:806.*

37. Ohashi, Y., S. Motojima, T. Fukuda, and S. Makino. 1992. Airway hyperresponsiveness, increased intracellular spaces of bronchial epithelium, and increased infiltration of eosinophils and lymphocytes in bronchial mucosa in asthma. *Am. Rev. Respir. Dis. 145:1469.*
38. Durham, S. R., D. A. Loegering, S. Dunnette, G. J. Gleich, and A. B. Kay. 1989. Blood eosinophils and eosinophil-derived proteins in allergic asthma. *J. Allergy Clin. Immunol. 84:931.*
39. Humbles, A. A., D. M. Conroy, S. Marleau, S. M. Rankin, R. T. Palframan, A. E. Proudfoot, T. N. Wells, D. Li, P. K. Jeffery, D. A. Griffiths-Johnson, et al. 1997. Kinetics of eotaxin generation and its relationship to eosinophil accumulation in allergic airways disease: analysis in a guinea pig model in vivo. *J. Exp. Med. 186:601.*
40. Gonzalo, J. A., C. M. Lloyd, D. Wen, J. P. Albar, T. N. Wells, A. Proudfoot, A. C. Martinez, M. Dorf, T. Bjerke, A. J. Coyle, and J. C. Gutierrez-Ramos. 1998. The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. *J. Exp. Med. 188:157.*
41. Gereda, J. E., D. Y. Leung, A. Thatayatikom, J. E. Streib, M. R. Price, M. D. Klinnert, and A. H. Liu. 2000. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet 355:1680.*
42. Michel, O., J. Kips, J. Duchateau, F. Vertongen, L. Robert, H. Collet, R. Pauwels, and R. Sergysels. 1996. Severity of asthma is related to endotoxin in house dust. *Am. J. Respir. Crit. Care Med. 154:1641.*
43. Peden, D. B., K. Tucker, P. Murphy, L. Newlin-Clapp, B. Boehlecke, M. Hazucha, P. Bromberg, and W. Reed. 1999. Eosinophil influx to the nasal airway after local, low-level LPS challenge in humans. *J. Allergy Clin. Immunol. 104:388.*
44. Tulic, M. K., J. L. Wale, P. G. Holt, and P. D. Sly. 2000. Modification of the inflammatory response to allergen challenge after exposure to bacterial lipopolysaccharide. *Am. J. Respir. Cell Mol. Biol. 22:604.*