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Temporal Role of Chemokines in a Murine Model of Cockroach Allergen-Induced Airway Hyperreactivity and Eosinophilia[1]

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The increase in inner-city asthma among children appears to be due to allergic responses to several allergens. Recent studies have demonstrated that Ags derived from cockroaches are especially prominent in these settings and a significant health concern for the induction of asthma in children. In the present study, we have outlined the development of a murine model of cockroach allergen-induced airway disease and assessed specific mechanisms of the response, which resembles atopic human asthma. The allergic responses in this model include allergen-specific airway eosinophilia and significantly altered airway physiology, which directly correlates with inflammation. We have further utilized this allergen to establish primary and secondary rechallenge stages of late phase hyperreactivity exacerbation. This latter stage is characterized by greater changes in airway physiology than the primary stage, and it is likely due to the preexisting peribronchial inflammation present at the time of the second allergen rechallenge. We have identified specific roles for CC chemokines during these stages, with MIP-1α being an important eosinophil attractant during the primary stage and eotaxin during the secondary rechallenge stage. The development of these models allows the evaluation of mediators involved in both stages of cockroach allergen challenge, as well as the testing of specific therapeutic modalities. ***The Journal of Immunology,*** **1998, 161: 7047–7053.**

Morbidity due to asthma continues to rise, especially among inner-city children. Recent data indicates that the disproportionate increase in densely populated urban areas appears to be due to allergen exposure. In particular, allergies to cockroach Ag have demonstrated a significant correlation to the rise of adolescent asthma in the more densely crowded inner cities, where large numbers of cockroaches can be found (1–3). Although the increase in asthma in inner cities cannot be linked solely to cockroach Ag, as increases in other allergens such as dust house mite have also been detected, 60% of inner city asthmatics have highly elevated IgE levels specific for cockroach Ags. Furthermore, some studies have suggested that the prevalence of sensitivity to cockroach Ags may be comparable with that for mite or cat allergens among acute asthmatics (4). Such patients are most often found in crowded, lower socioeconomic areas where dwellings are commonly infested with cockroaches (5). Constant exposure to threshold amounts of specific cockroach allergen establishes a persistent inflammatory response within the airway (4, 6).

In order to elucidate the specific responses that are associated with cockroach Ag sensitivity, our laboratory has developed a murine model of cockroach allergen-induced hyperreactive airway inflammation and we have begun to assess specific mechanisms that are involved in different stages of this response. Previous data in guinea pigs have shown that aerosolized cockroach Ag can be utilized to induce airway inflammation and alter airway physiology (7). Development of mouse models of allergic airway inflammation with altered physiology will allow better characterization and identification of inflammatory molecules to be targeted for therapy during the responses. In addition, the use of mouse models allows access to a wide range of reagents and genetically altered animals for the investigation of specific responses and molecules.

The data in the present study establishes a murine model for examining specific stages of inflammatory responses induced by cockroach allergen characterized by a significant eosinophilic influx and concurrent airway hyperreactivity. In addition, we have established models of primary challenge and secondary rechallenge stage allergic exacerbations, which elicit different responses in the absence or presence of preexisting eosinophilic inflammation, respectively. In order to elucidate the mechanisms that mediate these two distinct stages, we have investigated the role of selected CC chemokines. Chemokines are a large family of inducible proteins classified into two main subfamilies based on the position of cysteine residues (8). The CC chemokines have been extensively studied in association with allergic inflammation by virtue of their preferential recruitment of eosinophils, monocytes, basophils, and a variety of T cell subsets. We report the differential role of eotaxin and MIP-1α,[3] two potent murine eosinophil attractants (9, 10), in the primary vs rechallenge stages of cockroach Ag-induced allergic airway inflammation. The identification of mediators pivotal to the development of airway inflammation and hyperreactivity to this allergen may aid our understanding of the pathology of asthma and serve as a preclinical model for testing reagents.

Materials and Methods

Animals

Female C57/BL6 mice were purchased from either The Jackson Laboratory, (Bar Harbor, ME) or Charles River Breeding Laboratories (Wilmington, MA) and were maintained under standard pathogen-free conditions.

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[3] Abbreviations used in this paper: MIP-1α, macrophage inflammatory protein-1α; BAL, bronchoalveolar lavage; EPO, eosinophil peroxidase; hpf, high power fields; NRS, normal rabbit serum.

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All materials were obtained from Sigma Chemical Company (St. Louis, MO) unless otherwise indicated.

Sensitization and induction of the airway response

Normal C57/BL6 mice were immunized with 10 μg of cockroach allergen (Bayer, Elkhart, IN) in IFA on day 0. In order to localize the response to the lung, the mice were given an intranasal administration of 10 μg of cockroach allergen in 10 μl of diluent, on day 14. This initial intranasal Ag induced little cellular infiltrate into the lungs of the mice upon histological examination. Mice were then challenged 6 days later (referred to hereafter as primary challenge response) by intratracheal administration of 10 μg of cockroach allergen in 50 μl of sterile PBS or with PBS alone (vehicle). The magnitude of leukocyte recruitment in both the vehicle control and cockroach allergen-challenged mice was examined histologically. Only the cockroach allergen-challenged mice displayed a significant inflammatory response that included mononuclear cell and eosinophil infiltration. Some mice were given a second intratracheal injection of either cockroach allergen (10 μg in 50 μl) or diluent control and subsequently analyzed (secondary rechallenge response). In separate studies, the effect of anti-murine MIP-1α and anti-murine eotaxin polyclonal Abs on cockroach Ag-induced responses were assessed by giving sensitized mice an i.p. dose of the Ab (0.5 ml, titers of 10^6/ml) at 1 h prior to each Ag challenge. Normal rabbit serum (NRS) was used as a control. Polyclonal Abs had previously been demonstrated to block the chemotaxis of murine eosinophils *in vitro* (data not shown).

Morphometric analysis of airway and peribronchial eosinophil accumulation

To assess migration of eosinophils into the airway, we subjected the mice to a 1 ml bronchoalveolar lavage (BAL) with PBS containing 25 nM EDTA at various time points postchallenge. The cells were then dispersed using a cytospin (Shandon Scientific, Runcorn, UK) and differentially stained with Wright-Giemsa stain. The cell types (mononuclear phagocytes, lymphocytes, neutrophils, and eosinophils) were expressed as a percentage based on 200 total cells counted/sample.

Lungs from mice immunized and challenged with cockroach allergen or vehicle were preserved with 4% paraformaldehyde at various time points postchallenge. The fixed lungs were embedded in paraffin and multiple 3-μm sections cut and then differentially stained with Wright-Giemsa for the identification of eosinophils and viewed at 1000×. The individual eosinophils were counted from 100 high powered fields (hpf) per lung at each time point using multiple step sections of lung. The eosinophils counted were only in the peribronchial region; this assured the enumeration of only those eosinophils within or immediately adjacent to an airway. The inflammation observed in this model was completely associated with the airway with little or no alveolitis.

Quantitation of chemokines by ELISA

The levels of chemokine protein in whole lung homogenate were measured by specific ELISA using a modification of a double-ligand method as previously described (11). Briefly, lung tissue was homogenized on ice using a tissue-tearer (Biospec Products, Racine, WI) for 30 s in 1 ml of PBS containing 0.05% Triton X-100. The resulting supernatant was isolated following centrifugation (10,000 × *g*). To measure MIP-1α and eotaxin-1 (hereafter referred to as "eotaxin") levels in this supernatant, flat-bottom 96-well microtiter plates (Nunc Immunoplate 1 96-F, Roskilde, Denmark) were coated with 50 μl/well of rabbit anti-MIP-1α or anti-eotaxin 1 polyclonal Abs for 16 h at 4°C and then washed with PBS and 0.05% Tween-20. Nonspecific binding sites were blocked with 2% BSA in PBS and incubated for 90 min at 37°C. Plates were rinsed four times with wash buffer and cell free supernatants were added (neat and 1/10) followed by an incubation for 1 h at 37°C. Plates were washed four times, streptavidin-peroxidase conjugate (Bio-Rad, Richmond, CA) was added, and the plates were incubated for 30 min at 37°C. Plates were washed again and chromogen substrate (Bio-Rad) was added and incubated at room temperature to the desired extinction. The reaction was terminated with 50 μl/well of 3 M H_2SO_4 solution and the plates were read at 490 nm in an ELISA reader. Standards were 0.5 log dilutions of murine rMIP-1α or eotaxin from 1 pg/ml to 100 ng/ml. ELISAs for these chemokines did not cross-react with each other, JE, MARC, murine MCP-5, IL-6 or murine TNF.

Measurement of eosinophil peroxidase (EPO)

Cell-free BAL supernatants were analyzed for EPO as a marker of eosinophil degranulation. A total of 50 μl of each BAL sample were mixed with 100 μl of substrate (0.2 mg/ml *o*-phenylenediamine in Tris, pH 8, containing 0.1% Triton and 0.02% H_2O_2) in 96-well microtiter plates (Nunc-Immunoplates I 96-F). The reaction was allowed to progress for 30 min before quenching with 50 μl of 4 M sulfuric acid. Plates were read at 490 nm using an ELISA reader.

Measurement of airway hyperreactivity

Airway hyperreactivity was measured using a Buxco mouse plethysmograph, which is specifically designed for the low tidal volumes (Buxco, Troy, NY), as previously described (12). Briefly, the mouse to be tested was anesthetized with sodium pentobarbital and intubated via cannulation of the trachea with an 18-gauge metal tube. The mouse was subsequently ventilated with a Harvard pump ventilator (tidal volume = 0.4 ml, frequency = 120 breaths/min, positive end-expiatory pressure 2.5 to 3.0 cm H_2O) and the tail vein was cannulated with a 27-gauge needle for injection of the methacholine challenge. The plethysmograph was sealed and readings were monitored by computer. Since the box was a closed system, a change in lung volume was represented by a change in box pressure (P_{box}), which was measured by a differential transducer. The system was calibrated with a syringe that delivered a known volume of 2 ml. A second transducer was used to measure the pressure swings at the opening of the trachea tube (P_{aw}), referenced to the body box (i.e., pleural pressure), and to provide a measure of transpulmonary pressure ($P_{tp} = P_{aw} - P_{box}$). The trachea transducer was calibrated at a constant pressure of 20 cm H_2O. Resistance is calculated by the Buxco software by dividing the change in pressure (P_{tp}) by the change in flow (F) ($\delta P_{tp}/\delta F$; units = cm H_2O/ml/s) at two time points from the volume curve, based upon a percentage of the inspiratory volume. Once the mouse was hooked up to the box it was ventilated for 5 min prior to acquiring readings. Once baseline levels were stabilized and initial readings were taken, a methacholine challenge was given via the cannulated tail vein. After determining a dose-response curve (0.001 to 0.5 mg), an optimal dose was chosen (0.1 mg of methacholine), which was used throughout the rest of the experiments in this study. After the methacholine challenge, the response was monitored and the peak airway resistance was recorded as a measure of airway hyperreactivity.

Statistics

Statistical significance was determined by ANOVA with p values <0.05.

Results

Inflammatory responses during cockroach allergen challenge

The sensitization of mice with cockroach allergen systematically over a 14-day period was accomplished by immunization with cockroach allergen in IFA. Localization of the response to the airway was mediated by a single intranasal administration at day 14, followed by an intratracheal primary challenge at day 20. Histological analysis of the ensuing inflammation demonstrated a significant influx of leukocytes around the airways (Fig. 1, *A* to *C*). This peribronchial inflammation consisted of mononuclear cells (monocytes and lymphocytes) and eosinophils at all time points examined. Subsequent studies focused on the effect of cockroach allergen rechallenge on airway inflammatory responses. An increased severity of this peribronchial eosinophilia was observed during the secondary rechallenge vs primary cockroach Ag intratracheal administration at each time point examined (Fig. 1, *D* to *F*). Quantitation of the kinetics of this inflammation indicated that eosinophils were elevated around the airways at 8 h after primary challenge compared with vehicle-challenged controls and continued to intensify, peaking between 24 and 48 h and declining by 72 h postchallenge (Fig. 2). However, following secondary allergen rechallenge, the number and rate of eosinophil accumulation rose steeply, increasing sixfold over primary challenge levels by 24 h (1581 ± 641 vs 261 ± 40 cell/100 hpf, respectively). We also characterized the influx of eosinophils into the airway by BAL leukocyte analysis. The eosinophil influx into the airway was detected at 8 h after primary allergen challenge (9 ± 2.5% of total BAL leukocytes, using $n = 6$ animals), persisted at 24 h (16.2 ± 2.4%), and peaked by 48 h postchallenge (34.6 ± 8%). No time-dependent changes in eosinophil accumulation were observed in the BAL in cockroach Ag-sensitized, vehicle-challenged mice (3.5 ± 0.6% at 24 h after primary challenge). Secondary rechallenge with cockroach Ag further increased the proportion of eosinophils in the BAL, accounting for 40 ± 7% of total

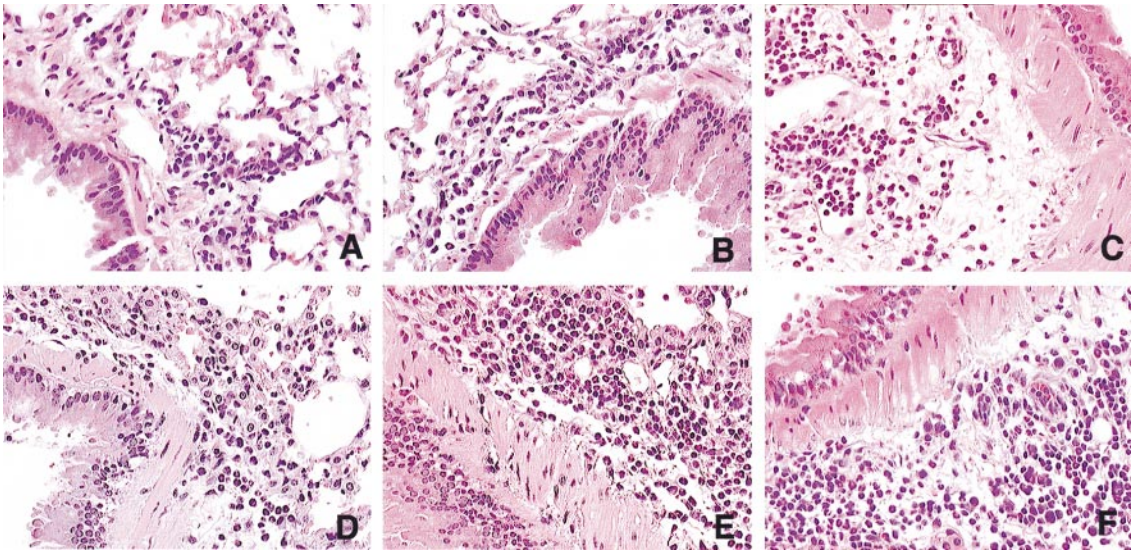

FIGURE 1. Hematoxylin and eosin-stained sections of lung from mice sensitized and challenged with cockroach Ag. *Upper panel* shows representative lungs at 8 h (*A*), 24 h (*B*), and 48 h (*C*) after primary challenge while *lower panel* shows lungs at 8 h (*D*), 24 h (*E*), and 48 h (*F*) after secondary rechallenge.

leukocytes ($n = 8$ animals) at 24 h, and 80 ± 2.2% by 48 h. This response would appear to by synergistic rather than simply additive of two primary challenge responses. Altogether, these data demonstrate that a primary allergen challenge of sensitized mice elicits a peribronchial inflammatory response, characterized by significant eosinophil accumulation. However, subsequent challenges with the same Ag greatly increase the rate and severity of this inflammation.

Induction of airway hyperreactivity following cockroach allergen challenges

The assessment of reversible airway hyperreactivity responses is a clinically relevant marker of asthmatic airway disease. Allergen-challenged mice were examined for changes in airway physiology at various time points (8, 24, and 48 h) after primary challenge, (Fig. 3). Vehicle-challenged control mice had virtually no increase in airway resistance, as compared with background resistance, when given i.v. methacholine. In contrast, cockroach allergen-sensitized mice demonstrated significant increases in airway resistance at 8 and 24 h after cockroach Ag challenge upon methacholine administration. By 48 h after allergen challenge, the airway resistance changes had diminished.

Rechallenging sensitized mice with a second exposure of cockroach Ag, at 48 h after primary Ag challenge, allowed analysis of lung function at a point when significant peribronchial inflammation was still present, but airway hyperreactivity was decreasing (Fig. 3). At 24 h after secondary rechallenge (72 h after primary challenge), the airway resistance response was significantly increased compared with those animals that received vehicle upon

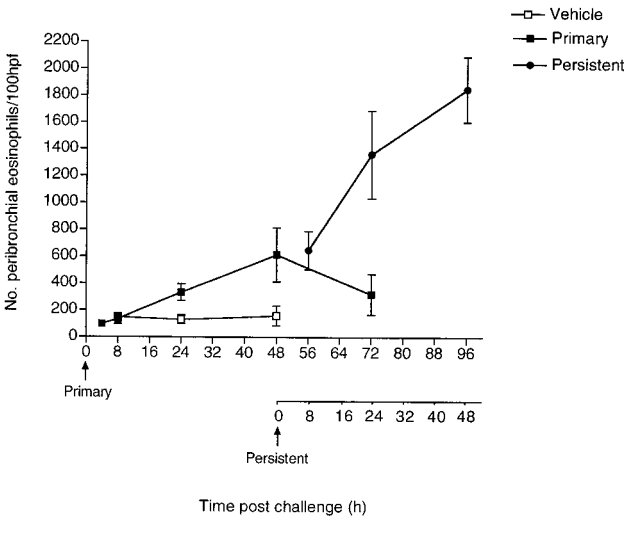

FIGURE 2. Sensitized mice challenged once with cockroach Ag. ($t = 0$ h) developed marked peribronchial eosinophilia compared with time-matched vehicle-challenged controls. Secondary rechallenge with cockroach Ag at $t = 48$ h exacerbated the eosinophilia in a time-dependent manner. Numbers represent means ± SE of eosinophils counted per 100 hpf magnification, ×1000) using $n = 6$–9 animals/group.

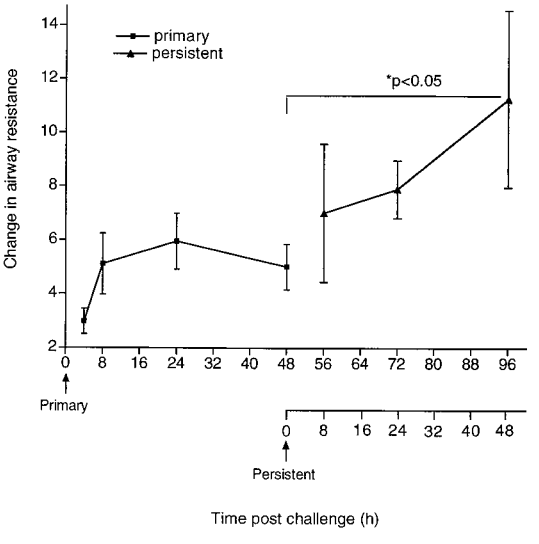

FIGURE 3. Changes in lung function in sensitized mice at various times following primary ($t = 0$ h) or secondary ($t = 48$ h) cockroach Ag challenge. Changes in hyperreactivity were significantly different ($*p < 0.05$) at 48 h after rechallenge (i.e., 96 h following the primary challenge) compared with 48 h after primary challenge. Points represent mean change in resistance (units = cm H_2O/ml/s) ±SE using $n = 6$–9 animals/group.

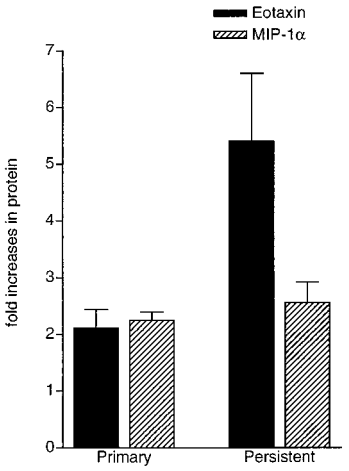

FIGURE 4. MIP-1α and eotaxin protein levels in whole lung homogenates increased at 8 h following a single cockroach Ag challenge, while only eotaxin increased at 8 h during the secondary rechallenge response. Values present mean fold increases $\pm$ SE over mean levels for controls (sensitized, saline-challenged animals) using n = 3–5/animals treatment group. Control levels of MIP-1α and eotaxin were 0.88 $\pm$ 0.3 ng/ml and 25.3 $\pm$ 1.9 ng/ml, respectively.

secondary rechallenge (7.5 $\pm$ 0.8 vs 3.5 $\pm$ 1.2, respectively). Furthermore, the airway hyperreactivity response was greater in the rechallenged animals compared with the primary stage, at similar time points postchallenge (Fig. 3). Interestingly, unlike the primary challenge, airway resistance continued to increase by 48 h during the secondary rechallenge response. Since a concurrent increase in the severity of the airway inflammation was observed in the rechallenge vs primary responses, these studies indicate that airway physiological responses correlate with the intensity of the inflammation within the airway, in this model.

The role of the MIP-1α and eotaxin in primary and rechallenge allergen exacerbations

The chemokines eotaxin and MIP-1α have been demonstrated to be potent eosinophil chemoattractants (9, 10) and we wished to assess their role in our model. Analysis of whole lung homogenates by specific ELISA revealed that levels of both chemokines were maximal at 8 h after primary challenge. However, levels of eotaxin, but not MIP-1α, were significantly higher in the secondary rechallenge compared with the primary challenge lungs (Fig. 4).

Pretreatment of sensitized mice with polyclonal neutralizing Abs to mMIP-1α prior to cockroach Ag challenge decreased the peribronchial eosinophilia at 24 h following primary challenge, but were ineffective at inhibiting the infiltration after secondary allergen rechallenge (Fig. 5*A*). In contrast, Abs to eotaxin inhibited eosinophilia by 60% in the secondary rechallenge but not primary stage (Fig. 5*B*). Interestingly, no significant differences were observed in peripheral blood eosinophil numbers following pretreatment with anti-MIP-1α prior to primary Ag challenge or anti-eotaxin prior to secondary rechallenge compared with NRS controls. Furthermore, Abs raised against eotaxin (but not MIP-1α) were able to significantly attenuate airway hyperresponsiveness during the secondary rechallenge response compared with normal rabbit serum controls. Neither Ab significantly effected hyperresponsiveness during the primary challenge (Fig. 6).

We also examined the activation state of the eosinophils during the secondary rechallenge phase by analysis of the EPO content in the BAL. Time-dependent changes in BAL EPO content following Ag challenge were observed, with peak production at 8 h (Fig. 7). At this time point, however, BALs from animals rechallenged with cockroach Ag contained significantly more EPO than those that had undergone primary challenge alone. Furthermore, the rechallenge EPO levels could be attenuated by pretreatment of the mice with anti-eotaxin, but not anti-MIP-1α, Abs before each challenge (Fig. 8).

Discussion

In these studies, our laboratory has described a murine model of cockroach allergen-induced airway hyperreactivity. In this model, the responses resemble those that have been observed in human asthmatic patients (i.e., airway eosinophilia and airway hyperreactivity) (13). In addition, our studies have outlined models of both primary challenge and secondary rechallenge exacerbation of the cockroach allergen-induced eosinophil accumulation, and we present evidence that these are differentially mediated by the chemokines MIP-1α and eotaxin. Only anti-eotaxin Abs were able to significantly affect changes in airway physiology associated with the secondary rechallenge response. Prior to primary cockroach allergen challenge, there is little inflammation present in the airways of these allergic mice and a significant increase in both mononuclear cells (monocytes and lymphocytes) and eosinophils

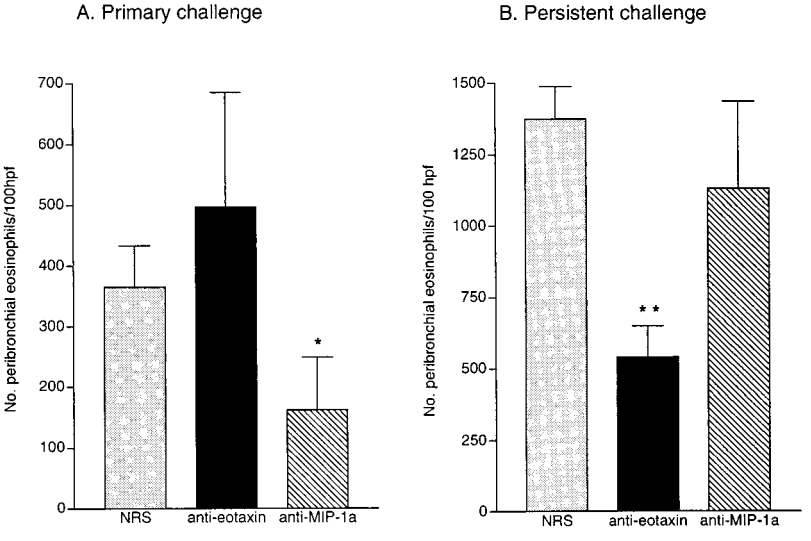

FIGURE 5. Different chemokines mediate peribronchial eosinophil accumulation following primary and secondary cockroach Ag challenges (at 24 h). Anti-MIP-1α Abs given 1 h prior to single challenge blocked eosinophilia while anti-eotaxin Abs blocked only eosinophilia following secondary rechallenge compared with NRS controls. All points represent mean $\pm$ SE eosinophils counted per 100 hpf; (magnification, $\times$1000) for n = 7–9 animals/group. $*p < 0.05$; $**$, $p < 0.01$.

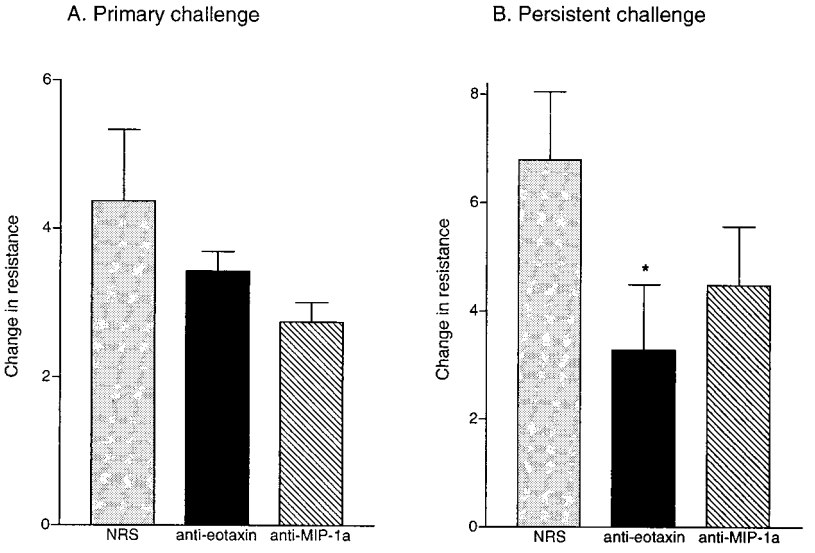

FIGURE 6. Pretreatment of sensitized mice with anti-MIP-1α or anti-eotaxin Abs at 1 h prior to a single cockroach Ag challenge did not appear to significantly attenuate hyperresponsiveness compared with NRS controls. However, anti-eotaxin Abs were able to significantly decrease airway hyperactivity following allergen rechallenge. All points represent mean ± SE change in resistance for $n = 7$–9 animals/group. *, $p < 0.05$.

occurs after the allergen challenge. When these animals are again rechallenged at this time with a second allergen stimulation, there is a resurgence of eosinophil accumulation in the BAL, which occurs more quickly and is associated with increased airway hyperreactivity. Our results suggest that the response elicited by sensitized subjects toward a single Ag exposure appears to resolve comparatively rapidly. However, persistent/repeated exposures in close succession appear to increase the extent and rate of the eosinophilia around the asthmatic airway, leading to prolonged airway hyperreactivity. Therefore, the more inflammation that is present prior to allergen challenge, the more severe the subsequent response.

Several animal models of allergic airway inflammation, including those that have utilized parasitic Ags (14), OVA (15), and plant extracts (16) as allergens, have characterized many of the basic

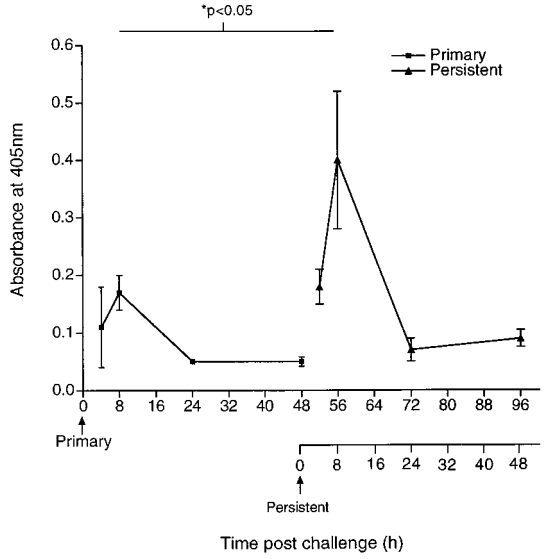

FIGURE 7. Temporal changes in eosinophil peroxidase levels in BAL from sensitized mice following primary challenge ($t = 0$ h) or secondary rechallenge ($t = 48$ h) with cockroach Ag. Significant differences (*, $p < 0.05$) were observed at 8 h after primary challenge compared with 8 h after rechallenge (i.e., 56 h post primary challenge). Absorbance levels of EPO in the BAL from sensitized, vehicle-challenged (8 h) animals were 0.14 ± 0.02. All points represent mean ± SE for $n = 4$–5 animals/group.

processes that occur during allergic airway responses. Animal models have delineated and described very important mechanisms of activation, however, they have either utilized a primary allergen exacerbation or have given repeated doses of the allergen over an extended period of time to the animal. In both cases very little information has been described comparing and contrasting the responses that are elicited in these two different stages (primary vs rechallenge) of allergic models.

Our murine model of cockroach antigen-induced allergic airway inflammation demonstrates key differences in the roles played by MIP-1α and eotaxin following primary and rechallenge exacerbations of this clinically relevant Ag. We have previously demonstrated that MIP-1α is a key murine eosinophil chemoattractant in a murine model of parasite Ag (*Schistosoma mansoni* egg Ag)-induced allergic inflammation (10), and it would appear that this chemokine plays a similar role following primary cockroach Ag challenge. However, physiological studies demonstrate that MIP-1α plays a minimal role in airway hyperreactivity in either allergic airway model. In contrast, eotaxin appears to be an important eosinophil attractant in the secondary rechallenge but not the primary response. This latter chemokine has also been characterized as a potent murine eosinophil attractant in both in vitro and in vivo experimental settings (10, 17, 18). However, our results are the first to demonstrate a role of endogenous eotaxin in airway physiological changes after allergen challenge.

The observations of differential chemokine roles could be explained by a number of interrelated mechanisms. First, although temporal increases in chemokine levels in whole lung homogenates are similar for both eotaxin and MIP-1α, the compartmentalized production by particular cell types and the timing of this production may be pivotal in determining the principle chemokine at work. For example, MIP-1α is produced by macrophage populations while the epithelium is an important source of eotaxin (19), thus enabling eosinophils to migrate into the interstitial compartment and accumulate around the airways. In additional studies in our laboratory, we have examined eosinophil degranulation in vitro and observed that eotaxin, but not MIP-1α, can induce murine eosinophil degranulation (data not shown). Since a number of investigators have demonstrated a direct correlation between eosinophil degranulation and airway function (20, 21), this may explain the ability of anti-eotaxin Abs to attenuate hyperreactivity only during the secondary rechallenge phase, when eosinophils are already present in the airway. Conceivably, however, it is possible

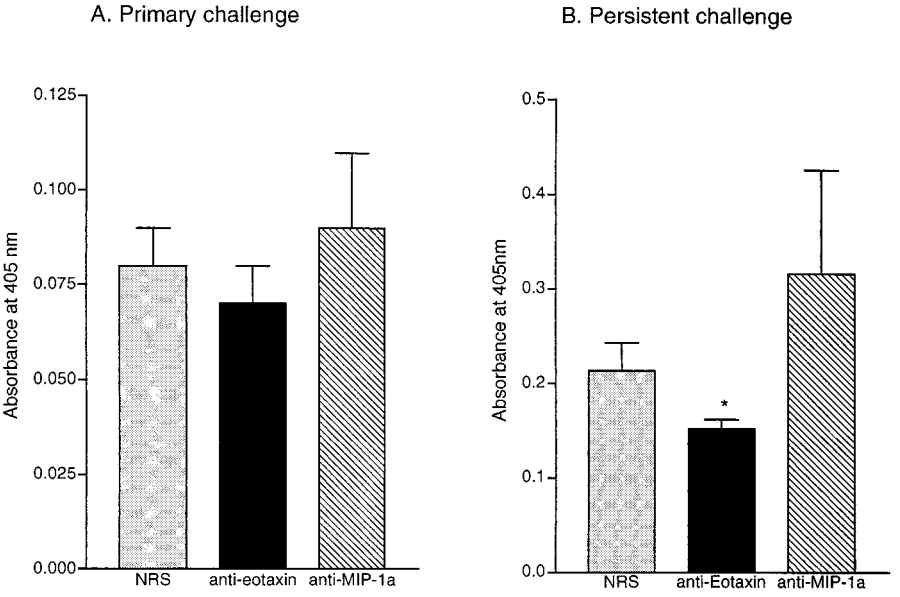

FIGURE 8. Effect of anti-chemokine Abs on EPO levels in the BAL of sensitized mice following cockroach Ag challenge. Neither Ab decreased EPO levels following primary challenge (*A*); however, following secondary rechallenge (*B*), anti-eotaxin Abs significantly reduced EPO levels compared with NRS controls. All points represent mean ± SE for $n = 7$–9 animals/group. *, $p < 0.05$.

that some component of the observed deterioration in lung function following secondary rechallenge may also be due to increased physical congestion of the airways through an increased inflammation.

Second, the relative contributions of different chemokines may reflect changes in the temporal expression of their receptors. Murine MIP-1α binds both murine eosinophil chemokine receptors, CCR1 and, to a lesser extent, CCR3, while eotaxin binds only to CCR3 (17, 22, 23). It is tempting to speculate whether events that promote the preferential binding of MIP-1α to CCR1 might occur in the primary phase, but shift to eotaxin and CCR3 following secondary allergen rechallenge. This latter aspect would be heightened by the significant increase in eotaxin, but not MIP-1α, during the secondary rechallenge response.

Finally, other groups (24, 25) have demonstrated that eotaxin is a potent local recruiter of eosinophils, but IL-5 is required to induce the systemic release of eosinophils from the bone marrow. Our observations would be compatible with this process in which a Th2-type response is implicated, and IL-5 is likely present to induce the systemic release of sufficient eosinophils, which can be subsequently recruited into the lung. In addition, IL-5 has been demonstrated to be an important priming agent for eosinophils toward other chemokines (26), although it remains to be established as to whether this cytokine potentiates the chemoattractant/degranulation activity of eotaxin. The net result of the above mechanisms is a greater number of leukocytes participating in the allergen-induced inflammatory response during the secondary rechallenge, resulting in severe airway inflammation. This was demonstrated by greater numbers of eosinophils migrating into the airway as well as the exacerbation of the hyperreactivity in the secondary rechallenge response compared with the primary stage allergen challenge.

The data presented in these studies have outlined a novel murine model for a clinically relevant and environmentally detrimental allergen from cockroaches. In addition, two distinct stages of disease have been examined, a primary allergen challenge stage, which elicits a response in the absence of significant inflammation, and a rechallenge stage, which induces a greater airway hyperreactive response in the presence of intense inflammation. It appears that the increased hyperreactivity responses during repeated allergen exposure is dependent upon eotaxin production, eosinophil accumulation, and degranulation. These studies further demonstrate the complexity of the allergic airway response and chemokine biology.

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