A murine model of allergic rhinitis: Studies on the role of IgE in pathogenesis and analysis of the eosinophil influx elicited by allergen and eotaxin

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Background: Allergic rhinitis is a prevalent disease with significant morbidity. Studies of its pathophysiology in human subjects have been limited. Nasal biopsy specimens are difficult to obtain, and nasal secretions incompletely reflect the cellular and molecular events in the mucosa. IgE-mediated mast cell activation and the elaboration of factors promoting eosinophil development and chemotaxis are likely to participate in pathogenesis.
Objectives: We sought to develop a murine model of allergic rhinitis, to use it to assess the role of IgE in pathogenesis, and to study the effects of IL-5 and eotaxin in the nasal mucosa.
Methods: A protein extract of *Aspergillus fumigatus* (*Af*) was instilled intranasally in mice. Histologic changes were examined in wild-type and IgE-deficient ($IgE^{-/-}$) animals. The effect of eotaxin administration was assessed in wild-type and IL-5 transgenic mice.
Results: *Af*-treated mice developed a nasal mucosal eosinophil influx comparable to that described for humans. This histology was distinct from that observed in a murine model of *Af*-induced asthma. The pathology appeared over a time course similar to that reported for human subjects. There was no difference in the intensity of the mucosal inflammatory infiltrate of *Af*-treated $IgE^{-/-}$ mice compared with wild-type mice. Eotaxin was able to recruit eosinophils to the mucosa but only in IL-5 transgenic animals.
Conclusion: We describe a murine model for allergic rhinitis with an eosinophilic infiltrate comparable to that found in human disease and have demonstrated that rhinitis can arise in the absence of IgE. We have shown that the eosinophil influx can be induced by eotaxin in the presence of IL-5. (J Allergy Clin Immunol 1998;102:65-74.)

Key words: *Allergic rhinitis, IgE, IL-5, eotaxin, mouse model*

Allergic rhinitis is a disorder with increasing prevalence in the Western world.[1-3] It is often debilitating and can lead to complications, including sinus disease, sleep disorders, and asthma flares. Allergic rhinitis is characterized histologically by a marked increase in eosinophils in the nasal submucosa and epithelium.[4-6] Increased numbers of epithelial mast cells have been seen in studies of seasonal allergic rhinitis, but this finding has not been consistently observed after local allergen challenge.[4,6]

It has been hypothesized that the recruitment of eosinophils to the allergic mucosa arises from the combined action of a number of cellular and molecular signals. These include IL-5 (derived from mast cells, T cells, and eosinophils), which drives their development and activation. Additional factors include the coordinated expression of the adhesion molecule very late activation antigen 4 (VLA-4) by eosinophils and its receptor vascular cell adhesion molecule 1 (VCAM-1) by the vascular endothelium of allergic tissues and the local elaboration of specific chemotactic factors, including eotaxin.[7-16]

The majority of patients with allergic rhinitis have high levels of allergen-specific IgE. A major role for IgE in pathogenesis is widely accepted, and the demonstration of allergen-specific IgE by skin testing or in vitro methods is often used both to confirm the diagnosis of allergic rhinitis, as well as to guide treatments, including environmental modification and immunotherapy.[17] IgE-producing plasma cells have been identified in the submucosal tissue of the nose.[18] It has been thought that the recurrent immediate and late-phase allergic reactions induced by IgE-dependent mast cell activation trigger a cascade, which leads to chronic allergic inflammation.[19,20] In addition to activating mast cells, preformed allergen-specific IgE has been postulated to facilitate antigen uptake and processing by B cells, leading to potent and persistent IgE responses during chronic allergen exposure, as well as the spread of allergies in a *multi-allergy syndrome*.[21-25] Some animal models of lower

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Abbreviations used
Af: *Aspergillus fumigatus*
ANOVA: Analysis of variance
IgE$^{-/-}$: IgE-deficient
NS: Normal saline

airway allergy have indicated a requirement for IgE in allergen-induced eosinophilic inflammation.[26-28]

Many studies of the pathogenesis of allergic rhinitis have been based on the analysis of nasal secretions as an indicator of changes occurring after exposure to allergen.[29-31] Recently, it has been shown that alterations in nasal secretions do not entirely reflect those that occur in the mucosa.[32] In an alternative approach, histologic examination of nasal mucosal biopsy specimens has been performed in atopic individuals with or without allergen stimulation.[4,6,32,33] The expression of mediators, including eotaxin, has been shown to be increased after allergen exposure in such mucosal samples.[34] The frequency of sampling human tissue is limited, and only small specimens, which may not be representative, can be obtained.

In view of the limitations with human subjects, animal studies of allergic rhinitis would provide some clear advantages. An animal model would allow for less restricted sampling of tissue after completely standardized sensitization and challenge protocols. Mouse models of human disease have recently become very desirable. A large number of mouse mutants with defects in factors thought to be involved in pathogenesis have been generated by gene targeting.[35] In addition, transgenic mice are available for studies on the effects of overexpression of some of the same molecules. Finally, some nongenetic approaches are most easily applied in mice. Blocking antibodies have been made for many cell surface and secreted murine molecules. Recombinant murine cytokines and chemokines have also been produced. The administration of these antibodies and factors during disease induction can be used to assess their roles in pathogenesis.

Existing mouse models for sensitivity to airborne allergens include asthma[36-40] and allergic conjunctivitis.[41] Although models of allergic rhinitis have been established in the guinea pig[42,43] and the rat,[44] a mouse model has not previously been reported. Here we describe a novel murine model for allergic rhinitis. To induce rhinitis, we sensitized mice by repeated nasal application of a protein extract of the naturally occurring airborne mold allergen, *Aspergillus fumigatus* (*Af*). The nasal mucosa of sensitized mice was examined by light microscopy and subjected to histomorphometric analysis. The animals had rhinitis with a marked infiltration of the nasal mucosa by eosinophils. We used the model, along with IgE-deficient (IgE$^{-/-}$) mice we had previously generated,[45] to investigate the role of IgE in this allergen-driven influx of eosinophils. *Af*-treated IgE-deficient mice displayed the same degree of eosinophilic inflammation as did wild-type controls, indicating the presence of IgE-independent pathways for the induction of allergic cellular infiltrates in the mouse nasal mucosa. To investigate the effects of an eosinophil-specific chemokine, eotaxin, in the nasal mucosa, we instilled recombinant eotaxin intranasally into wild-type and IL-5 transgenic animals. Although eotaxin did evoke significant vascular margination of eosinophils in both wild-type and IL-5 transgenic mice, a mucosal infiltrate of eosinophils was elicited only in IL-5 transgenic mice. This observation indicates that eotaxin-driven tissue influx of eosinophils occurs in the presence of IL-5. We believe that this model will prove to be useful in the further molecular and cellular analysis of the pathogenesis of allergic rhinitis.

METHODS

Reagents and mice

A mixture of culture filtrate and mycelial extracts of *Af* was kindly provided by Bayer Pharmaceuticals (Spokane, Wash.) and was diluted to a concentration of 2 mg/ml with normal saline. IgE-deficient mice were derived as previously described.[45] They were maintained inbred in a 129/SvEv background. Wild-type 129/SvEv mice were obtained from Taconic (Germantown, N.Y.) and housed in pathogen-free conditions in the Children's Hospital animal facility until they were 6 to 10 weeks of age. IL-5 transgenic mice were kindly provided by Dr. Colin Sanderson.[46] Control CBA/CaJ mice were obtained from the Jackson Laboratories (Bar Harbor, Me.). All mouse studies were done in accordance with Children's Hospital, Boston Animal Care and Use Committee guidelines, which are in accordance with the National Institute's of Health *Guide for the Care and Use of Laboratory Animals*, (NIH publication No. 86-23, as revised). Recombinant murine eotaxin was synthesized as described previously.[14,47]

Sensitization of mice and collection of specimens

Sensitization of mice to allergen was performed as previously described.[39] Mice were lightly anesthetized by Metofane inhalation (methoxyfluorane; Pittman-Moore, Mundelein, Ill.), and 100 μg (50 μl) of *Af* antigen or 50 μl of normal saline (NS) was applied to the left nare by using a micropipette with the mouse held in the supine position. After instillation, mice were held upright until alert. Mice were immunized three times a week for 3 weeks. Twelve hours after the final sensitizing dose, mice were again lightly anesthetized, and peripheral blood was obtained by retroorbital bleed. They were then euthanized by carbon dioxide inhalation.

Time course analysis

Mice were sensitized for 3 weeks according to standard protocol and were kept for 26 days without allergen exposure. At this time, histologic examination showed that the inflammatory infiltrate in the nasal mucosa had regressed. Mice were subsequently challenged with a single dose of *Af* antigen and euthanized at 0, 1, 4, 8, 12, 16, or 24 hours after challenge. As controls, mice were used that had not been exposed to the 3-week sensitization protocol. These control mice were killed at the same time points after a single exposure to A*f* antigen.

Eotaxin challenge

Purified recombinant eotaxin was prepared as previously described.[47] Mice were lightly anesthetized with metofane, and 50 μl NS containing 0, 0.5, or 5 nmoles eotaxin was applied to the nares with a micropipettor. The mice were held upright until alert. Four hours after treatment, they were euthanized, and the nasal mucosa was analyzed.

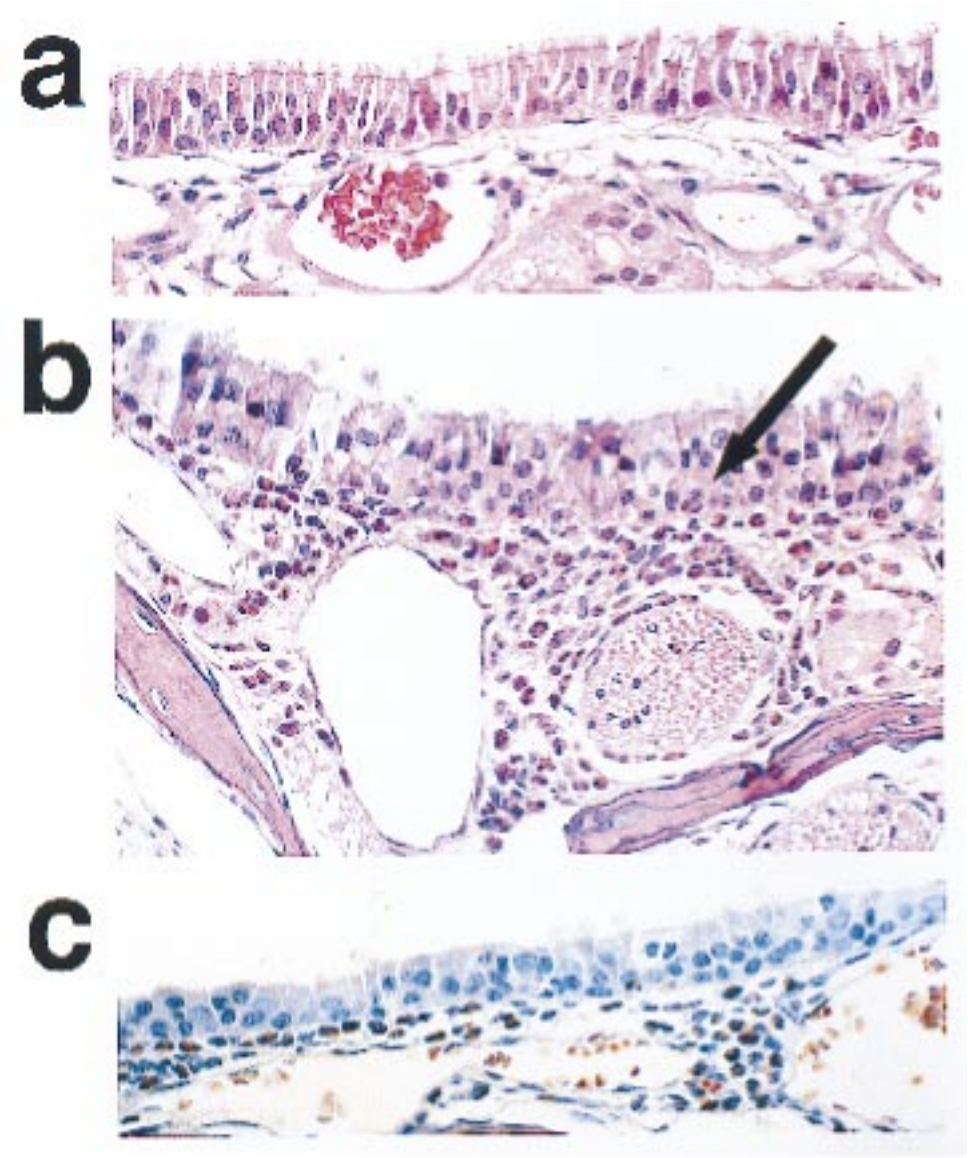

FIG. 1. Eosinophil influx into nasal mucosa of allergen-exposed mice. **A,** Nasal mucosa after exposure to normal saline shows rare submucosal eosinophils. **B,** After exposure to *Af* allergen, abundant eosinophils are seen in submucosa and in nasal epithelium *(arrow)*. **C,** Luna stain confirms that most inflammatory cells in *Af*-exposed animals are eosinophils. There were four animals in each treatment group. Sections were prepared and examined from each animal, and representative sections are shown. Original magnification, ×400.

Histologic analysis

After fixation for 24 hours in 10% neutral-buffered formalin, the skulls were skinned and incubated in decalcifying medium (Decalcifier B; Accra Lab, Swedesboro, N.J.) for 24 hours. Frontal cross sections, each taken at the same distance from the tip of the nose and containing the nasal cavity, were taken from each head. The sections were next embedded in paraffin, and 5 μm sections were cut. The inflammatory infiltrate was examined by using standard light microscopy of hematoxylin- and eosin-stained sections. In addition, the Luna stain was used to confirm the presence of eosinophils in the inflammatory infiltrate. For Luna stains, sections were incubated for 5 minutes in 0.9 volume Weigert's Iron Hematoxylin (0.005% acid hematoxylin and 0.6% ferric chloride in 2% HCl) with 0.1 volume of 1% Biebrich Scarlet and 0.1% Acid Fuchsin in 1% acetic acid. After differentiation in 1% acid alcohol, slides were washed in water. Final color development was done in 0.5% lithium carbonate. This stain is specific for eosinophils and renders their cytoplasm red-brown on a blue background.[48]

Histomorphometry

To quantitate the intensity of the infiltrate, total intravascular eosinophils and total eosinophils present in the epithelium and submucosa were counted in full cross sections of nasal mucosa. The cross sections were taken at the same anatomic site. For the time course experiment, cross sections from individual mice at seven different time points (14 mice total) were counted. For quantification of the infiltrates in IgE-deficient mice and after eotaxin exposure, cross sections from three individual mice were counted per group (total of 12 mice per experiment), and the results were averaged. Counting

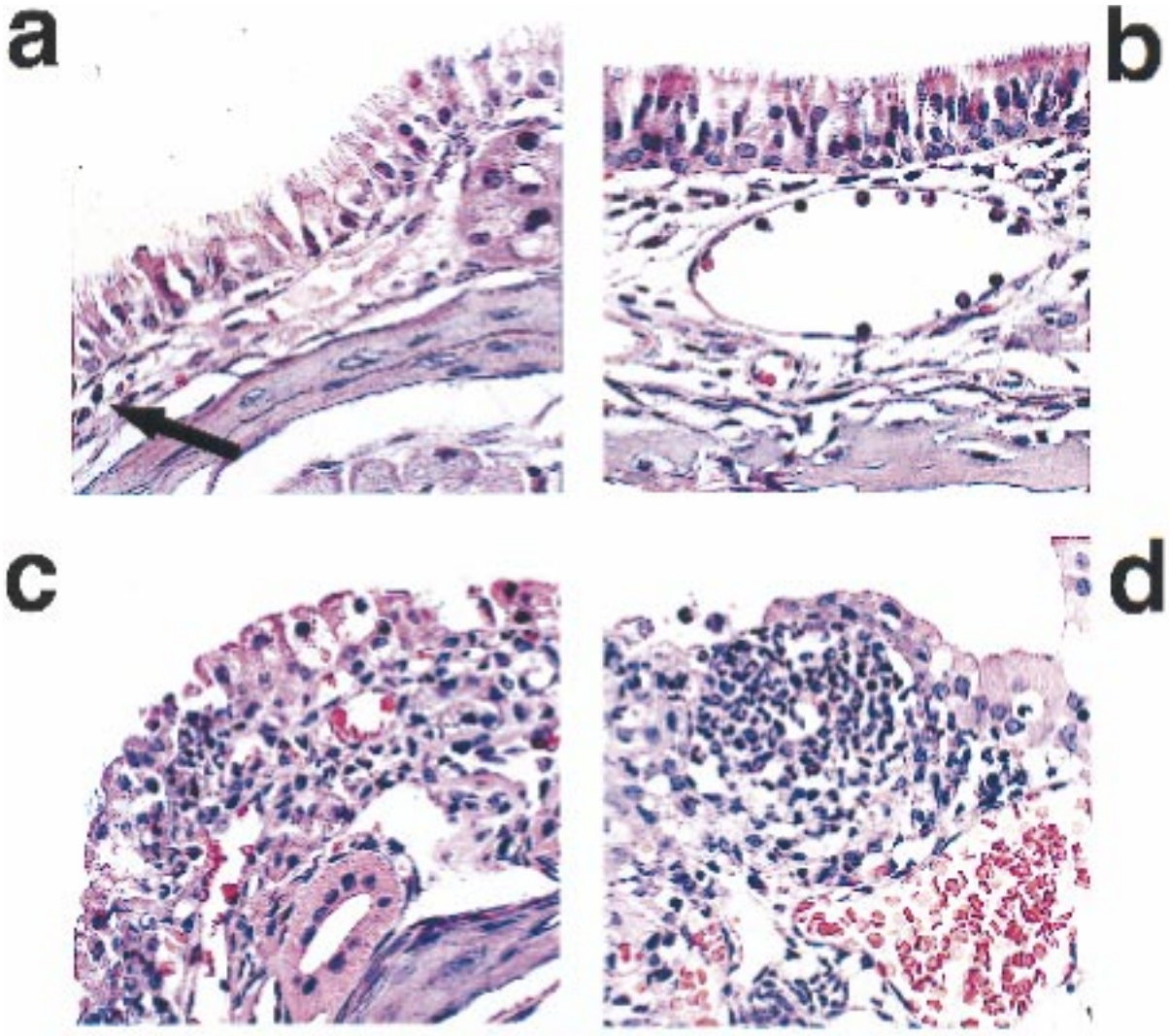

FIG. 2. Acute nasal mucosal response to allergen in previously-sensitized animals. **A,** Twenty-six days after completion of 3-week course of inhaled sensitization to *Af,* nasal mucosa has returned to normal, with rare intravascular and submucosal eosinophils *(arrow).* **B,** Four hours after reexposure to *Af,* numerous eosinophils are seen to marginate in submucosal vessels. **C,** After 8 hours, moderate eosinophilic infiltrate is seen in submucosa, with involvement of epithelium. **D,** Infiltrate peaks at 12 hours after reexposure. Original magnification, ×400.

was performed on slides that were unidentified as to the immunization protocol used or the genetic status of the mouse. To test the validity of the counting protocol, a single slide was counted four times, showing good reproducibility. Morphometric data for eosinophil counts in the noses of NS- and *Af*-treated mice were square root–transformed and tested for significance with Student's *t* test (two-sample, two-tail). Time course eosinophil counts were analyzed by using analysis of variance (ANOVA) (two-factor without replication). Eotaxin dose responses in wild-type versus IL-5 transgenic mice were also compared with ANOVA (two-factor with replication).

Quantification of serum IgE levels

Serum IgE levels were determined by ELISA as described in the Pharmingen (San Diego, Calif.) protocol. Briefly, 96-well plates were coated overnight with monoclonal anti-mouse IgE antibody (clone R35-118, 2 μg/ml; Pharmingen, San Diego, Calif.) and then blocked with phosphate-buffered saline/3% bovine serum albumin before application of samples and serially diluted purified mouse IgE standard in triplicate. After overnight incubation, biotinylated monoclonal anti-mouse IgE antibody (Clone R35-72, 2 μg/ml; Pharmingen, San Diego, Calif.) was added and allowed to incubate for 45 minutes before addition of horseradish peroxidase–avidin (2.5 mg/ml) (Zymed, San Francisco, Calif.). After 30 minutes, substrate solution, consisting of equal quantities of the 50× concentrate solutions of 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and hydrogen peroxide (Zymed) was added to the wells. The reaction was allowed to proceed for 20 minutes before reading in a Pasteur Diagnostics optical densitometer (Kallestad Diagnostics, Chaska, Minn.) at 405 nm.

RESULTS

Nasal mucosal response to allergen exposure

We and others have previously shown that intranasal administration of *Af* evokes an intense eosinophil-pre-

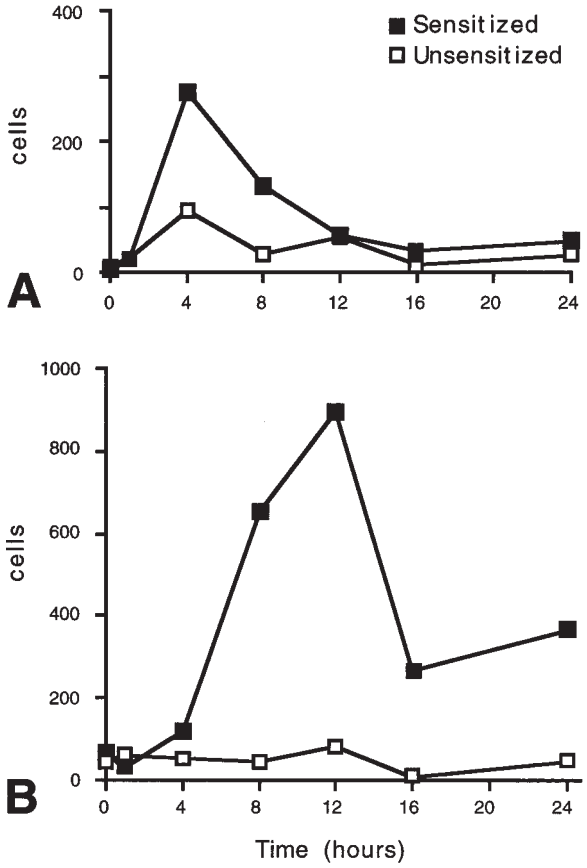

FIG. 3. Time course of nasal eosinophil influx after *Af* challenge in previously sensitized mice. Mice intranasally sensitized over 3 weeks with *Af* were allowed to rest for 26 days. After rechallenge with *Af*, mice were killed at 0, 1, 4, 8, 12, 16, and 24 hours *(solid squares)*. Unsensitized mice challenged with single dose of *Af* were used as controls *(open squares)*. Each square represents one animal. **A,** Intravascular accumulation of eosinophils peaks at 4 hours. **B,** Eosinophils in submucosa and mucosa reach their maximum level after 12 hours. Two-factor ANOVA without replication was used to compare time courses of tissue eosinophil influx ($p = 0.027$) and intravascular accumulation ($p = 0.107$).

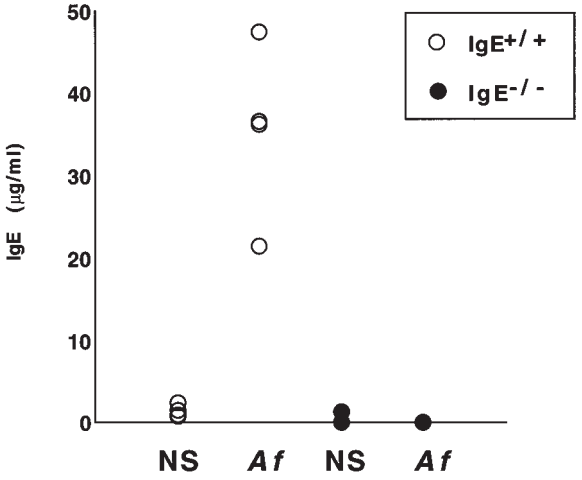

FIG. 4. IgE levels in *Af*-treated mice. Serum was prepared from wild-type (IgE$^{+/+}$, *open circles*, $n = 4$) and IgE-deficient (IgE$^{-/-}$, *solid circles*, $n = 4$) mice at completion of 3 weeks of intranasal *Af* sensitization. *Af* elicits vigorous IgE response in wild-type, but not IgE$^{-/-}$, mice. IgE levels were determined by ELISA.

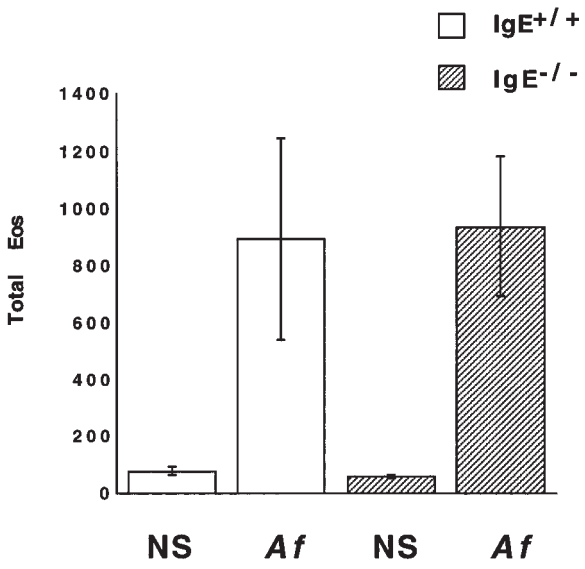

FIG. 5. Eosinophil counts in nasal mucosa of *Af*-treated mice. Eosinophils were counted in entire frontal nasal cross sections of wild-type (IgE$^{+/+}$) and IgE-deficient (IgE$^{-/-}$) mice after 3 weeks of intranasal *Af* sensitization. Data are presented as means ± SEM. Three mice were analyzed in each group. *Af* treatment induced nasal mucosal eosinophilia both in wild-type ($p = 0.024$) and IgE$^{-/-}$ mice ($p = 0.007$).

dominant reaction in the lungs, with peribronchial and perivascular pulmonary inflammation.[37,39,49] To determine whether allergic rhinitis was also induced in these animals, we examined nasal tissue from treated mice. Histologic sections of nasal mucosa from animals sham treated with NS showed intact ciliated epithelium overlying a submucosa in which only rare scattered inflammatory cells were seen (Fig. 1, *A*). As has been described for human subjects, focal squamous metaplasia was present in the ciliated columnar epithelium.[32] Scattered mast cells were present in the submucosa. In contrast, animals exposed to allergen (Fig. 1, *B*) showed dense inflammatory infiltrates in many areas of the submucosa with occasional cells involving the epithelium *(arrow)*. Almost all of the inflammatory cells had the eosinophilic granules and multilobed nuclei typical of murine eosinophils. These cells were stained by the Luna stain,[48] confirming their identity as eosinophils (Fig. 1, *C*). Morphometric analysis revealed that NS-treated mice had an average of 78 ± 25 eosinophils per full cross section of nasal mucosa. Many more eosinophils (888 ± 351) were present in the *Af*-exposed animals ($p = 0.02$). Neutrophils were identified only very rarely. These cells had a pale cytoplasm with no evident granules and were not stained with the

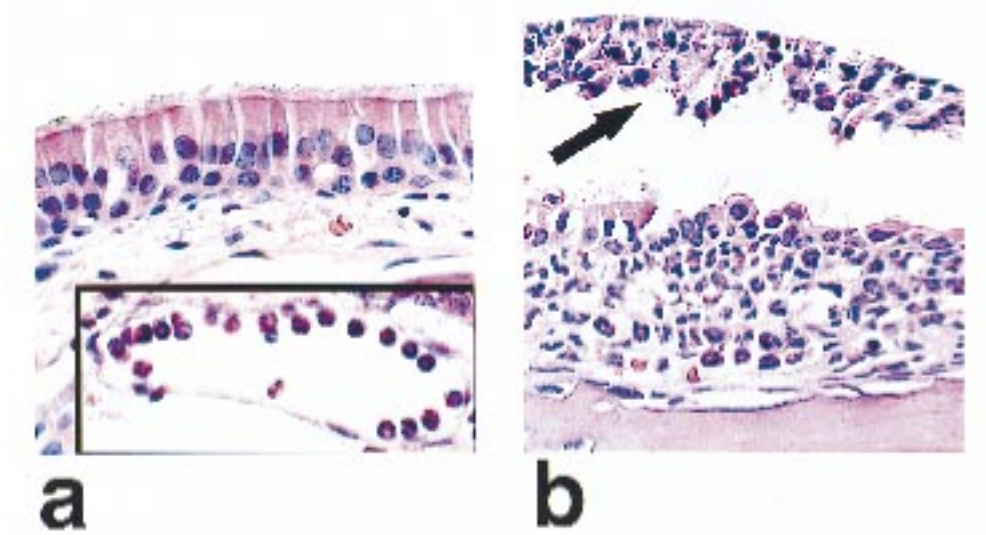

FIG. 6. Effects of topical eotaxin on nasal mucosa of wild-type and IL-5 transgenic mice. Purified recombinant eotaxin (5.0 nmoles) was instilled intranasally. Four hours after treatment, animals were euthanized, and nasal mucosa was analyzed. **A,** Control CBA mice show minimal eosinophil infiltrate after nasal exposure to eotaxin. *Inset,* Marginating eosinophils in a nasal mucosal venule of eotaxin-treated wild-type mouse. **B,** In an IL-5 transgenic mouse exposed to eotaxin, a dense inflammatory infiltrate is seen in submucosa and nasal epithelium, with many eosinophils in nasal secretions *(arrow).* Original magnification, ×400.

Luna stain. Rare mononuclear cells were also evident. This finding in the nasal mucosa contrasted with our previous observation in the lungs and bronchial mucosa of similarly treated mice in which significant numbers of mononuclear cells were admixed with the eosinophilic infiltrate.[39] No significant difference was seen in the number of mast cells present in the submucosa or mucosal epithelium of these animals when compared with NS-treated mice.

Time course of the inflammatory response

Allergic rhinitis can arise both in the setting of chronic allergen inhalation and after acute challenge in sensitized individuals. To characterize nasal mucosal changes after an acute allergen encounter, we examined *Af*-sensitized mice after a single intranasal *Af* dose. Animals sensitized to *Af* by inhalation for a period of 3 weeks, following our standard protocol, were allowed to rest for 26 days. After this period, the inflammatory infiltrates had disappeared (Fig. 2, *A*). Only rare eosinophils remained (see *arrow*), with a frequency indistinguishable from that found in untreated animals. Four hours after reexposure to allergen, a marked margination of eosinophils was seen in the submucosal blood vessels (Fig. 2, *B*). At this point, only small numbers of inflammatory cells had migrated into the submucosal tissue. Eight hours after challenge, large numbers of eosinophils were present in the submucosa (Fig. 2, *C*). The maximum infiltrate was observed after 12 hours. At this point, many inflammatory cells were present in the nasal epithelium, and associated epithelial damage was evident (Fig. 2, *D*).

The intensity of the eosinophilic infiltrate at each time point was quantified by histomorphometry. Intravascular eosinophils peaked 4 hours after reexposure to *Af* (Fig. 3, *A*), preceding the sub- and intramucosal eosinophil influx, which reached a maximum at 12 hours (Fig. 3, *B*). The data obtained from these counts was subjected to two-way ANOVA. This confirmed that the numbers of tissue eosinophils in *Af*-sensitized mice over this time course differed significantly from the controls ($p = 0.027$). The numbers of intravascular eosinophils over this time course were not shown to differ significantly by ANOVA between the test sample and controls ($p = 0.107$), but this may have been due to the small sample size and the fact that the numbers of intravascular eosinophils in sensitized mice rapidly returned to baseline after their peak at 4 hours.

The role of IgE in murine *Af*-induced allergic rhinitis

Elevated total and allergen-specific IgE levels are almost invariably present in patients with allergic rhinitis. Documentation of allergen-specific IgE is used in establishing the diagnosis. To assess whether IgE has an obligate role in the pathogenesis of allergic rhinitis in this murine model, we compared the responses of wild-type and IgE-deficient ($IgE^{-/-}$) mice. We had previously generated the $IgE^{-/-}$ mutants by targeted deletion of the Cε exons encoding the IgE heavy-chain constant-region domains. As a result of this genomic deletion, the mice are completely unable to produce IgE.[45]

Wild-type and $IgE^{-/-}$ mice were subjected to *Af* sensitization over 3 weeks. This treatment induced total IgE levels averaging 35.3 μg/ml in wild-type animals (Fig. 4). No IgE was detected in the mutants. We have previously shown that *Af*-specific IgE is induced (in wild-type animals only) by using this protocol.[39] The pathologic changes in the nasal mucosa of *Af*-treated $IgE^{-/-}$ mice were indistin-

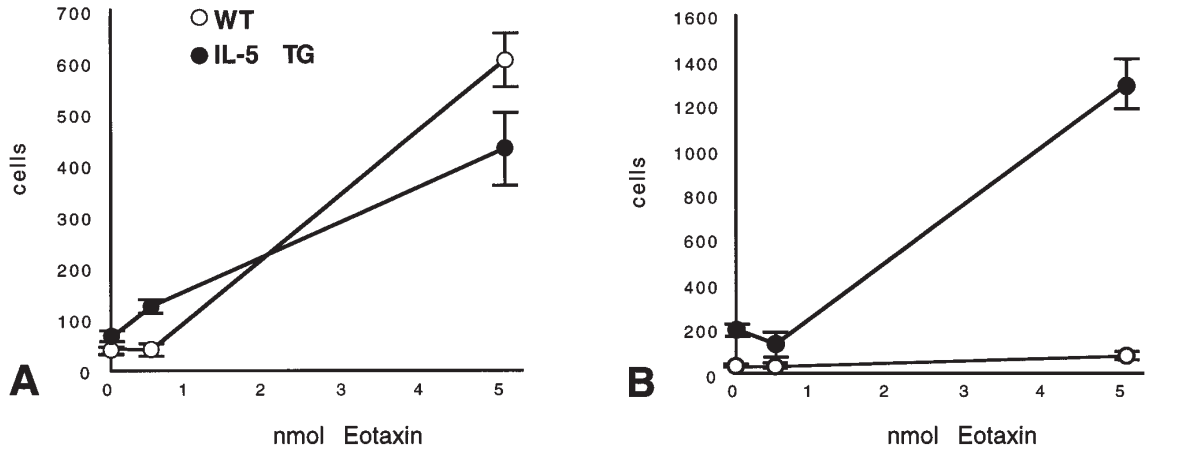

FIG. 7. Morphometric analysis of eosinophil infiltration after eotaxin exposure in wild-type and IL-5 transgenic mice. Eotaxin (0.5 nmol, 50 nmol, or vehicle alone) was applied to nares of wild-type and IL-5 transgenic mice (three per group), and 4 hours later, nasal mucosa was examined. **A,** Intravascular eosinophils. Wild-type *(open circles)* and transgenic *(solid circles)* mice show marked margination of eosinophils after high-dose eotaxin exposure. **B,** Mucosal eosinophils. Only in IL-5 transgenic mice is significant infiltration of mucosal and submucosal tissues seen. ANOVA (two factor with replication) demonstrated statistically significant differences in eotaxin dose-responsive eosinophil recruitment to tissue ($p < 0.0001$) and intravascular spaces ($p = 0.003$).

guishable on light microscopic inspection from those of wild-type animals. To confirm that the degree of eosinophil influx was identical in control and mutant mice, eosinophils were counted in entire cross sections of nasal mucosa (Fig. 5). Using this analysis, we determined that the mean total eosinophil number per cross section was 888 in *Af*-treated wild-type animals and 934 in identically treated $IgE^{-/-}$ mice. The response to *Af* was statistically significant in both wild-type and $IgE^{-/-}$ mice ($p = 0.024$ and 0.007, respectively). Taken together, these observations indicate that IgE is not required in this murine model for the initiation of the allergic inflammatory cascade, which leads to eosinophilic infiltration of the nasal mucosa.

Effects of topical eotaxin

The recruitment of eosinophils to allergic sites is likely to involve a sequence of events, including differentiation from bone marrow precursors, activation, adhesion to vascular endothelium, and chemotaxis to the locus of inflammation. The chemokine, eotaxin, has been identified as a particularly eosinophil-specific chemotactic agent and has previously been shown capable of inducing eosinophilic infiltration of pulmonary and cutaneous tissues.[14,15,50] To examine whether eotaxin could induce eosinophil migration into the nasal mucosa, we applied it intranasally. Our pilot studies, as well as some published work, had suggested that eotaxin would only act in vivo if a pool of IL-5–activated eosinophils was present in the circulation.[51,52] Therefore we investigated the ability of eotaxin to attract eosinophils to the nasal mucosa in wild-type and IL-5 transgenic mice. These transgenic mice have eosinophilia primarily limited to the hematopoietic organs.[46]

Sham-treated wild-type and IL-5 transgenic mice had only rare nasal mucosal eosinophils (data not shown). Eotaxin given to wild-type animals elicited only a minimal increase in submucosal inflammatory cells (Fig. 6, *A*). Surprisingly, despite the absence of significant peripheral blood eosinophilia ($< 10^5$/ml) in the wild-type mice, many eosinophils were seen to marginate in the submucosal blood vessels (Fig. 6, *A*, *inset*). In contrast to wild-type mice, eotaxin-treated IL-5 transgenic animals showed dense inflammatory infiltrates in the nasal mucosa with involvement of the nasal epithelium (Fig. 6, *B*) indistinguishable from the reaction of wild-type mice to allergen exposure. Casts of secretions containing abundant eosinophils were present in the lumen *(arrow)*.

The intensity of the eosinophilic infiltrate was quantified by counting intravascular and tissue eosinophils in full nasal cross sections (Fig. 7). Untreated animals had only a few intravascular eosinophils; 44 ± 11 cells for wild-type and 65 ± 11 cells for IL-5 transgenic mice. Eotaxin application led to a marked intravascular accumulation of eosinophils in both groups. Wild-type animals treated with 5 nm eotaxin had 604 ± 52 intravascular cells per cross section, and IL-5 transgenic mice had 430 ± 70 (Fig. 7, *A*). In contrast, a significant infiltration of the submucosal and mucosal tissues (1294 ± 113) was observed only in IL-5 transgenic mice (Fig. 7, *B*). Eotaxin-treated wild-type mice had only 77 ± 21 tissue eosinophils in the nasal mucosa. These results demonstrate that eotaxin is a potent inducer of eosinophil influx into the nasal mucosa. Although it is capable of causing intravascular eosinophil accumulation in the nasal mucosa of wild-type mice, eotaxin appears to require the additional presence of IL-5 to drive marginated eosinophils to migrate into the surrounding tissue.

DISCUSSION

Allergic rhinitis is a complex disease process involving the coordinated action of many elements of the immune

system.[16] Studies of allergic rhinitis in human subjects have relied on measurements of mediators of allergy in nasal secretions, after local application of allergen, or during seasonal fluctuations in allergen exposure.[29-31] Recently, it has been shown that the findings observed in nasal secretions do not completely reflect those seen in nasal mucosa.[32] For this reason, several investigations have been performed on nasal mucosal biopsy specimens obtained from human subjects.[4,6,32,33] However, studies of human subjects have some limitations. Only relatively small and often superficial areas of nasal mucosa are represented in biopsy specimens. In addition, the frequency of sampling is quite restricted, and there are constraints on biologic and pharmacologic manipulations.

By contrast, animal models allow examination of the entire nasal mucosa and submucosa after challenge with a wide variety of allergens and immune-response modifiers. Models of allergic rhinitis have previously been described in the guinea pig[42,43] and the rat.[44] A murine model of allergic rhinitis would be a particularly valuable new investigative tool. It would permit the analysis of the roles of a number of factors with putative roles in allergic pathogenesis by using the large number of strains now available with targeted mutations of the relevant genes. Studies in mice would also allow the analysis of a wide variety of biologically active materials and blocking agents that have been developed for this species. Models of responses to airborne allergens in mice have previously been reported for asthma[36-40] and, more recently, for conjunctivitis.[41] Here we describe a murine model for allergic rhinitis induced by inhalation of *Af* protein.

Kurup and colleagues[37,49,53-56] have previously shown that intranasal administration of *Af* protein leads to an eosinophilic inflammation of the lower airways. We have found that this is associated with the induction of airway hyperresponsiveness.[39] In this study we show that intranasal *Af* instillation also induces the characteristic histopathologic changes of allergic rhinitis. The paucity of mononuclear cells distinguished the nasal inflammation from the infiltrate we had previously characterized in the bronchial mucosa and lungs of allergen-exposed mice.[39] Whereas pulmonary inflammation induced by *Af* inhalation contained an admixture of eosinophils and mononuclear cells, the rhinitis elicited by the same treatment was characterized by a vast predominance of eosinophils.

The time course and cell types involved in this model are quite similar to that described in human subjects with allergic rhinitis. Repeated nasal exposure to *Af* resulted in a dense submucosal inflammatory infiltrate in which eosinophils predominated. In several areas the eosinophilic infiltrate was seen to involve the overlying epithelium, resulting in epithelial damage similar to what has been described in human mucosa.[4,6] In cases in which mucus secretions remained present in the material submitted for histology, eosinophils were the predominant cell type, similar to nasal secretions in human subjects.

There are some differences between the pathologic changes observed in this model and those observed in human subjects. One distinctive feature of the murine histology, both in sensitized and unsensitized mice, is that lymphocytes and neutrophils are very rare. In humans, mononuclear cells, the majority of which appear to be lymphocytes, are the predominant inflammatory cells seen in the submucosa of unstimulated subjects.[32] However, the total number of these $CD45^+$ mononuclear cells has been observed to be stable after stimulation by direct application of allergen, although shifts in CD4/CD8 ratios have been described.[6] Similarly, Igarashi et al.[33] found no significant differences in the number of B cells, T cells, or monocytes in the nasal mucosa of healthy individuals when compared with allergic patients. In contrast, a marked increase in eosinophils was seen in human nasal mucosa after allergen exposure,[6] and increased numbers of eosinophils were seen in patients with allergies compared with normal control subjects.[33] In addition, seasonal shifts in allergen levels are associated with dramatic changes in eosinophil counts[32] but are not accompanied by any significant fluctuations in lymphocytes or neutrophils.[4] In one study an increase in neutrophils was observed[6] in addition to a marked increase in eosinophils. The presence of neutrophils in that case may have occurred because antigen was directly applied and was only present for a period of minutes, in contrast to other reported studies, which investigated seasonal changes presumably with extended periods of inhaled allergen exposures.[4] These findings suggest a significant role for eosinophil infiltration in human subjects, with a lesser involvement of lymphocytes and neutrophils. The described findings, particularly in studies involving sustained or seasonal exposures to allergen, are quite comparable with the histopathologic changes in our model, which includes repeated applications of allergen over an extended period of time.

A time course experiment in our model shows a sequence of events similar to that described in human subjects. When mice that had been sensitized intranasally to allergen were maintained for 26 days in the absence of allergen, the nasal mucosal histology returned to normal, with only scant inflammatory cells present in the mucosa. Four hours after restimulation, marked margination of eosinophils in submucosal blood vessels was observed. Eight hours after stimulation, a dense inflammatory infiltrate was seen in the submucosa; the extent of this infiltrate peaked after 12 hours. Subsequent time points at 16 and 24 hours revealed a decrease in the intensity of the infiltrate. This sequence is similar to that described in human studies, in which the maximum count of eosinophils in nasal secretions occurred 9 to 11 hours after stimulation.[19]

The strong clinical association between IgE and human allergic rhinitis, the marked increase in IgE seen in wild-type mice after *Af* exposure, and the multiple established functions of IgE in initiating hypersensitivity reactions led us to investigate the role of IgE in nasal rhinitis. Several mouse models of asthma have previously suggested an obligate role for IgE in allergen-induced eosinophilic inflammation in the lungs.[26-28] Using IgE-deficient mice, however, we showed that the intense allergen-induced inflammation of the nasal mucosa was completely independent of IgE. This finding parallels our previous obser-

vation that *Af*-induced pulmonary inflammation is indistinguishable in IgE$^{-/-}$ mice and wild-type controls.[39]

The IgE-independent tissue eosinophilia we observed in both the nasal and bronchial mucosae could have arisen through a number of pathways. These include IgE-independent mast cell activation followed by late-phase reactions, as well as by T-cell hypersensitivity responses. It is well-established that mast cell activation can be triggered in the absence of IgE by IgG, complement anaphylatoxins (C3a and C5a), and various histamine-releasing factors.[45,57-60] Recurrent mast cell stimulation by these non-IgE factors during chronic allergen exposure could lead to the elaboration of mediators that drive a state of persistent late-phase reactions and chronic inflammation. However, allergic inflammation can be elicited even in the absence of mast cells.[28,38] In this case the response of T cells to mucosally encountered allergen could drive a state of eosinophilic inflammation independent of the existence of either IgE or mast cell–derived mediators.[61,62] It is clear that many factors can drive mast cell activation and eosinophilic inflammation and that multiple pathways can converge in the pathogenesis of allergic rhinitis.[16]

In our model the number of mast cells in the murine nasal submucosa was quite low, and no significant changes were seen before and after allergen stimulation. Given the fact that the early, but not the late, phase of allergic rhinitis is thought to be mast cell driven,[5] this is perhaps not surprising. The data on mast cell density in human nasal mucosa have been inconsistent. Some investigators observed no significant changes in the numbers of either mucosal or intraepithelial mast cells after direct stimulation by allergen-soaked paper filter disks.[6] In another study, submucosal mast cells did not vary during the allergy season, but intraepithelial mast cells did increase during the season.[4] A third study reported an increase in mast cells after allergen challenge.[63]

The finding that nasal mucosa eosinophilia is independent of IgE suggests that other local factors may be of great importance in the development of a nasal eosinophilic infiltrate. One factor known to act locally as a chemoattractant for eosinophils is eotaxin. To further characterize the ability of eotaxin to elicit eosinophilic infiltration of tissues in vivo, we exposed mice to intranasal eotaxin. Normal mice showed only a minimal increase in mucosal eosinophils but had significant numbers of eosinophils marginating in the submucosal blood vessels after eotaxin exposure. Only IL-5 transgenic animals, which have high levels of circulating IL-5, showed, after eotaxin exposure, an eosinophilic infiltrate similar in density to that seen in *Af*-treated animals. No significant numbers of other cell types were recruited. Burke-Gaffney et al.[64] had previously shown that eosinophils exposed to eotaxin show an increased adhesion to lung microvasculature endothelial cells in vitro. This correlates with the observed marked margination of eosinophils in wild-type mice after eotaxin exposure. The role of IL-5 in generating an eosinophilic infiltrate had previously been shown by others.[9] The cooperative nature of eotaxin and IL-5 seen in our model is similar to that reported in guinea pig[51] and mice airways.[47] Mould et al.[52] have shown that eosinophil migration to skin after eotaxin injection is absent in IL-5–deficient mice but can be restored by pretreatment of the animals with IL-5. IL-5 probably acts both by increasing the eosinophil pool and by affecting the responsiveness of eosinophils to eotaxin. Therefore eotaxin, in the presence of sufficient levels of IL-5, is able to incite a significant eosinophilic infiltrate in murine nasal mucosa. This may be of clinical interest because eotaxin-specific inhibitors may significantly reduce the eosinophilic infiltration of nasal mucosa.

We anticipate that the murine model of allergic rhinitis described here will be useful to explore the utility of such potential therapeutic interventions, as well as to continue to probe the basic pathophysiologic mechanisms of the disease.

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