

Allergens, IgE, mediators, inflammatory mechanisms

Quantitation of inflammatory cells in the nasal mucosa of patients with allergic rhinitis and normal subjects

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Background: The role of inflammatory cells at the local site of allergic inflammation in the nose is unclear.

Methods: Nasal biopsy specimens were obtained from 10 patients with symptomatic seasonal allergic rhinitis and 10 normal subjects. Freeze-dried paraffin-embedded sections were stained for mononuclear cells and eosinophils. Tissues in Carnoy's fixative were stained for mast cells.

Results: T cells were much more plentiful than B cells or macrophages, and no significant differences were found between the two groups in the number of T cells, T-cell subsets, B cells, and macrophages. However, the number of CD25⁺ cells (lymphocyte activation markers) and the number of eosinophils were significantly higher in the allergic group than in the control group. There were no significant differences between the two groups in the total mast cell number. However, mucosal type mast cells were slightly increased, and a higher ratio of mast cells were costained for IgE in the allergic group. IgE⁺ cells mostly costained for mast cell tryptase and did not costain for J chain.

Conclusions: These data suggest that unlike granulocytes, in some mononuclear cells qualitative, not quantitative, changes may be important in allergic rhinitis and that IgE may not be locally produced in the nasal mucosa. (*J ALLERGY CLIN IMMUNOL* 1995;95:716-25.)

Key words: Allergic rhinitis, nasal mucosa, immunohistochemistry, lymphocyte, mast cell, IgE

In contrast to nonatopic persons, patients with allergic rhinitis react to allergen exposure with sneezing, rhinorrhea, nasal congestion, and pruritus. Antigen challenge of the lungs, skin, or nose in allergic patients leads to mast cell degranulation, and released mediators cause various effects on blood vessels, exocrine glands, or nerve fibers to evoke the allergic symptoms.¹⁻³ The antigen chal-

Abbreviations used

APAAP:	Alkaline phosphatase anti-alkaline phosphatase
BM:	Basement membrane
IL:	Interleukin
MBP:	Major basic protein
MC _T :	Tryptase-positive, chymase-negative mast cells
MC _{TC} :	Tryptase-positive, chymase-positive mast cells

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lenge also induces an infiltration of granulocytes and mononuclear cells,⁴ and this inflammatory response is often associated with hypersensitivity to antigen and to nonspecific irritants.⁵ Various inflammatory cells are thought to play important roles in the pathogenesis of allergic rhinitis. T cells and other inflammatory cells produce a variety of

cytokines.⁶ Interleukin(IL)-3 is a mast cell growth factor and stimulates other hematopoietic cells.⁷ IL-4 is necessary for IgE production by B cells,⁸ whereas γ -interferon inhibits the IL-4-mediated B-cell isotype switch to IgE production.⁹ IL-5¹⁰ and granulocyte-macrophage colony-stimulating factor¹¹ affect eosinophil proliferation or activation, and eosinophil products cause mucus secretion¹² and vasoactive effects.¹³

There are a few reports in which cellular infiltrates in nasal mucosa were quantified in patients with allergic rhinitis with immunohistochemical techniques. Fokkens et al.¹⁴ demonstrated increased numbers of CD1+ cells and IgE+ cells in patients with grass pollen allergy in comparison with nonallergic patients. Bentley et al.¹⁵ showed elevated numbers of activated eosinophils and intraepithelial mast cells. Recently, we used a freeze-dried tissue preparation technique with nasal tissues from nonatopic subjects with sleep apnea to obtain well-preserved tissue structure for inflammatory cell staining with monoclonal antibodies.¹⁶ In this study we compared atopic subjects with symptomatic allergic rhinitis in their allergy seasons with nonatopic subjects by using biopsy specimens to determine whether mononuclear cells were changed quantitatively or qualitatively and whether those changes were also associated with changes in mast cell or plasma cell numbers.

METHODS

Study subjects

Ten patients with seasonal allergic rhinitis (6 men and 4 women; aged 30 to 52 years) and 10 normal control subjects (8 men and 2 women; aged 21 to 53 years) were recruited for nasal biopsy. Allergic status was determined by the history of seasonal rhinitis and a positive prick skin test reaction to grass, ragweed, or tree allergens. Biopsies of allergic rhinitis subjects were performed during the allergy season when patients were having symptoms. Nasal or orally inhaled cromolyn sodium or corticosteroids and oral corticosteroids were not allowed for 6 weeks before biopsy, and no subject had experienced a respiratory tract infection during the month before biopsy. Oral antihistamines were allowed.

Nasal biopsy technique

Nasal biopsy specimens were obtained from the anterior portion of the inferior turbinates. After local anesthesia had been induced with lidocaine and epinephrine injection, 3 to 5 mm specimens were taken with Takahashi's forceps. The study protocol was approved by The Institute Clinical Review Subpanel of the National Institute of Allergy and Infectious Diseases, and all subjects gave informed consent.

Preparation of tissues

Fresh unfixed specimens were immediately cut into pieces (1 to 2 mm in size). One piece of each specimen was snap-frozen with liquid nitrogen or with precooled (dry ice) 2-methylbutane. Frozen specimens were lyophilized at -45°C , 5×10^{-2} mm Hg for 24 hours.¹⁷ Freeze-dried tissues were then allowed to warm up to room temperature and were embedded in paraffin. The paraffin-embedded blocks were sliced in $4\text{ }\mu\text{m}$ sections, and mounted on gelatin-coated microscope slides. Sections were kept at 4°C until use. Another piece of each specimen was fixed with Carnoy's fixative for 4 to 16 hours, then washed with absolute ethanol. These fixed tissues were also embedded in paraffin, sliced in $4\text{ }\mu\text{m}$ sections, and kept at 4°C .

Immunohistochemical staining

Staining for mononuclear cells and eosinophils. Freeze-dried paraffin-embedded sections were stained for leukocyte markers whenever the primary antibodies were not applicable for fixed tissues. After being deparaffinized in xylene and lightly fixed with acetone, sections were sequentially incubated with mouse monoclonal antibodies raised against human leukocyte antigens (anti-major basic protein [MBP] antibody from Sera-Lab; Sussex, England; other antibodies from Becton-Dickinson; Mountain View, Calif.) at room temperature for 40 minutes and visualized by an alkaline phosphatase anti-alkaline phosphatase (APAAP) method¹⁸ (DAKO Corp., Carpinteria, Calif.). Positive cells were recognized by their Fast Red color. Slides were counterstained with Mayer's hematoxylin solution and mounted in glycerin-gelatin solution.

Staining for mast cells. Carnoy's fixed paraffin-embedded sections were sequentially double-stained for the mast cell proteases, tryptase and chymase¹⁹ (antibodies were raised at Virginia Commonwealth University). Sections were incubated with mouse monoclonal anti-chymase at 4°C overnight, then visualized by the biotin-streptavidin peroxidase method. The sections were further incubated with mouse monoclonal anti-tryptase at 4°C overnight and visualized with a direct immunalkaline phosphatase method. The chymase-positive cells were recognized by the brown color of 3-amino-9-ethylcarbazole, and the tryptase-positive cells were recognized by their Fast Blue color.

Staining for IgE-positive cells. Carnoy's fixed tissues were stained for IgE alone, double-stained for IgE and tryptase, or double-stained for IgE and immunoglobulin J chain. Sections were first stained for IgE with a peroxidase anti-peroxidase method²⁰ (DAKO Corp.) and then for tryptase (or without tryptase in the case of staining for IgE alone) with a direct alkaline phosphatase immunostaining technique. To perform the double-staining for IgE and J chain, sections were first stained for IgE with a direct peroxidase immunostaining technique (Nordic Immunological, Capistrano Beach, Calif.) and then for J chain (Nordic Immunological) with

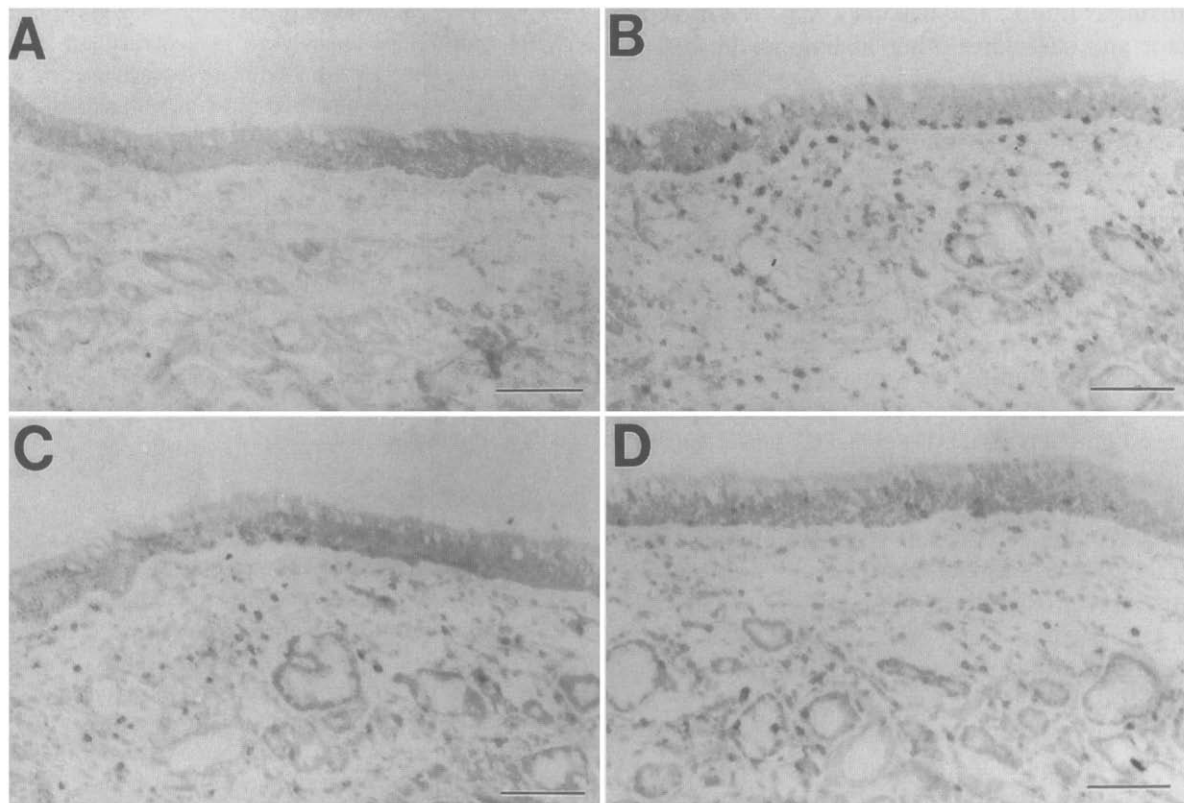


FIG. 1. Staining of nasal mucosa for T cells. Sections of freeze-dried paraffin-embedded nasal biopsy tissue from one of 10 patients with allergic rhinitis whose tissues were stained for (A) control (incubated with mouse IgG₁), (B) CD3, (C) CD4, and (D) CD8 with an APAAP technique. Scale bars indicate approximately 100 μ m.

an APAAP method. The IgE⁺ cells stained brown, and the tryptase-positive cells or the J chain positive cells stained blue. Changing the staining order (first tryptase or J chain, then IgE) did not affect the results.

Cell counting

Stained slides were coded, and cell counting was done by observers blinded to the diagnosis. Counting of stained cells was performed through an eyepiece grid at a magnification of $\times 450$. The scale of the grid was determined by an objective micrometer. The number of positively stained cells was counted in the lamina propria (within 200 μ m of basement membrane [BM]) along the entire BM in the section. The cell numbers were divided by the length of BM and expressed as the cell number per millimeter of BM.¹⁷ Because the epithelium was sometimes damaged and sufficient numbers of tissues suitable for the analysis of all cells of interest in the epithelium could not be obtained, only mast cells were enumerated in the epithelium. The number of intraepithelial mast cells was also expressed as the cell number per millimeter of BM. Variation of repeated count (intraobserver) was less than 5%.

Statistics

Because the cell numbers were not normally distributed, the two groups (allergy and control) were compared by the Mann-Whitney U test.

RESULTS

Mononuclear cells

Freeze-dried tissues were stained for lymphocytes and macrophages. Our previous experiments with surgically obtained nasal turbinates from patients with sleep apnea demonstrated that CD3⁺ cells (T cells) were abundant in the nasal mucosa, especially in the superficial lamina propria; whereas CD22⁺ cells (B cells) and CD14⁺ cells (monocytes/macrophages) were less plentiful.¹⁶ CD4⁺ cells in the nasal tissues were more plentiful than CD8⁺ cells in the lamina propria.¹⁶ Similar to our results with nasal turbinates, many CD3⁺ cells were present in the nasal mucosa of biopsy tissues obtained from atopic individuals (Fig. 1, B), and CD4⁺ cells were more marked than CD8⁺ cells (Fig. 1, C and D). There were fewer CD22⁺ cells

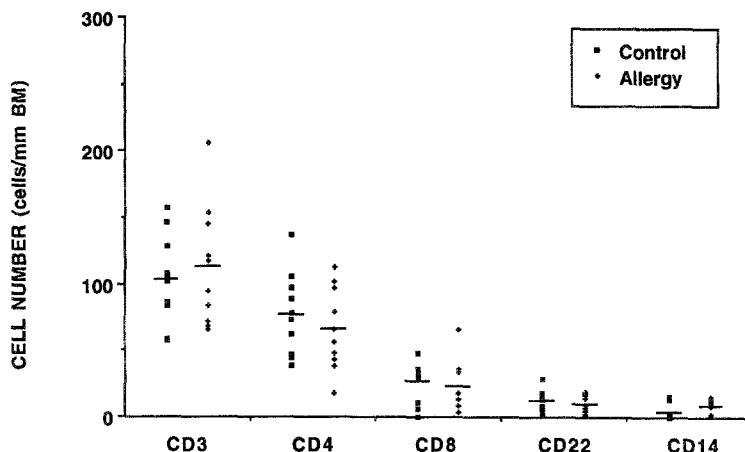


FIG. 2. Number of mononuclear cells in the nasal mucosa. Positively stained cells were counted in the superficial 200 μ m below the BM in the lamina propria and were divided by the length of the BM. Each point represents the result from an individual patient's tissue. Cross bars represent the mean. No significant differences between the allergic group and the control group (Mann-Whitney U test) were observed.

and CD14+ cells as compared with CD3+ cells in these allergic subjects (data not shown). When cell numbers were counted in the superficial lamina propria (within 200 μ m below the BM), atopic and control subjects' biopsy specimens contained 113 and 104 (mean value) CD3+ cells per millimeter of BM, respectively, whereas the numbers of CD22+ cells and CD14+ cells were about one tenth that of the CD3+ cells (Fig. 2). The numbers of CD4+ cells (66 and 77 cells/mm BM for the allergic patients and the control subjects, respectively) were about three times as large as those of CD8+ cells (23 and 26 cells/mm BM for the allergic patients and the control subjects, respectively). There were no significant differences between the allergic group and the normal group in the frequency of CD3+, CD4+, CD8+, CD22+, and CD14+ cells. However, cells staining for the lymphocyte activation marker, CD25 (α -chain of IL-2 receptor), were seen more frequently in many allergic subjects than in normal subjects (Fig. 3, A and B), and the difference between the two groups (9.0 cells/mm BM for the allergic group and 0.6 cells/mm BM for the normal group) was statistically significant ($p < 0.01$) (Fig. 4, A).

Eosinophils

Eosinophils were visualized in freeze-dried tissues by staining for MBP (Fig. 3, C and D). These MBP+ cells were seen as polymorphonuclear cells containing eosinophilic granules on the hematoxylin-eosin-stained sections of the same nasal tissues (data not shown). Eosinophils in the nasal mucosa

were found in groups in the lamina propria instead of being equally distributed throughout the tissue. Allergic subjects' tissues contained significantly more eosinophils than did normal subjects (9.1 and 1.5 cells/mm BM, respectively, $p < 0.001$; Fig. 4, B).

Mast cells

Mast cells were visualized in Carnoy's fixed tissues by staining for the neutral proteases, tryptase and chymase. Mast cells were evenly distributed throughout the tissue in the subepithelium and were also seen in the epithelium (Fig. 5, A and B). Intraepithelial mast cells were predominantly of the mucosal type (tryptase-positive, chymase-negative mast cells [MC_T]), whereas subepithelial mast cells were predominantly of the connective tissue type (tryptase-positive, chymase-positive mast cells [MC_{TC}]). Although some allergic tissue had many mast cells in the epithelium (Fig. 5, B), some allergic tissues also had few intraepithelial mast cells. The allergic group tended to have higher numbers of intraepithelial mast cells than the control group (mean value, 4.5 vs 1.3 cells/mm BM), although the difference was not significant (data not shown). In the subepithelium MC_T were somewhat more prominent in the allergy group than in the control group, especially in the superficial portion (mean value, 5.2 vs 2.5 cells/mm BM, $p < 0.05$) (Fig. 5, A and B and Fig. 6). The proportion of MC_T relative to total mast cells was also increased in the allergic patients as compared with normal subjects (26.4% and 7.4%, respectively, $p < 0.01$; Fig. 6). In contrast, neither the total number of mast cells

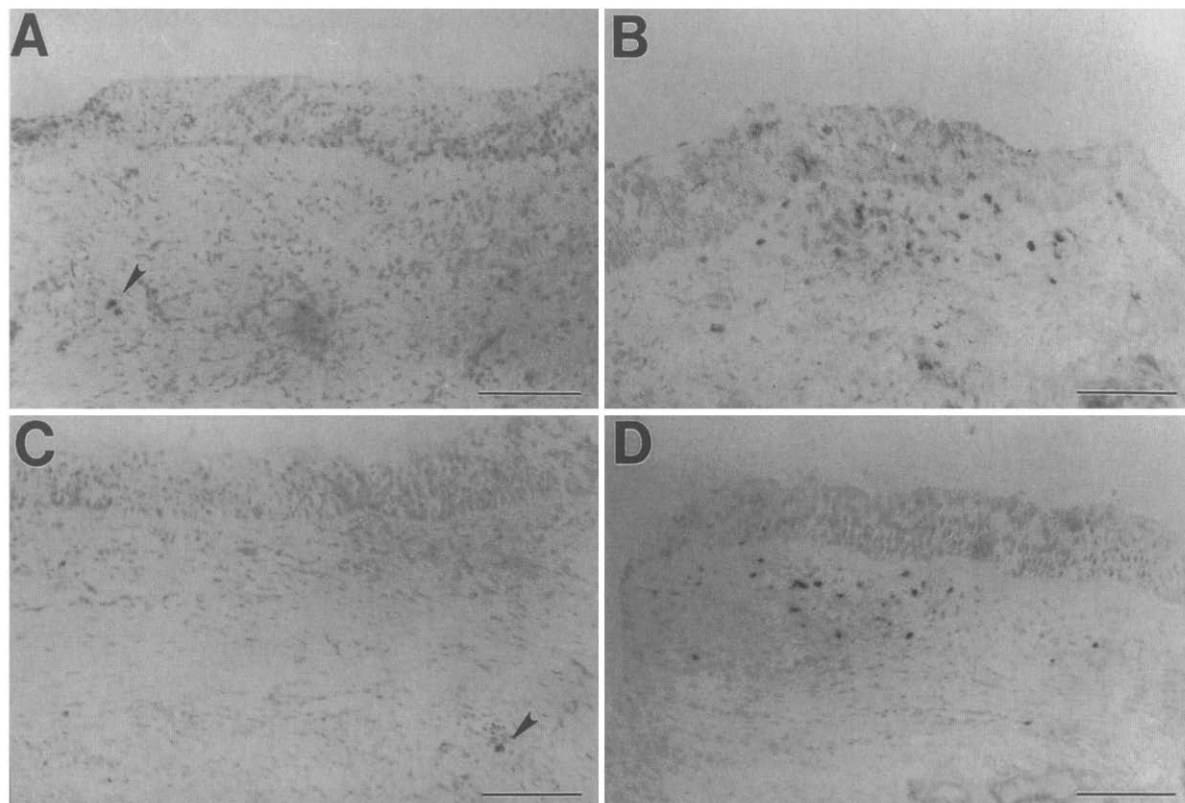


FIG. 3. Staining of nasal mucosa for CD25 and eosinophils. Sections of freeze-dried paraffin-embedded nasal biopsy tissue were stained for CD25 (**A** and **B**) and eosinophil MBP (**C** and **D**) with an APAAP technique. **A** and **C**, Biopsy specimen from a control subject. Positive cells are indicated by arrowheads. **B** and **D**, Biopsy specimen from a patient allergic rhinitis. Scale bars indicate approximately 100 μ m.

nor the number of MC_{TC} differed between the groups.

IgE+ cells

The serial sections of the Carnoy's fixed tissues were either stained for IgE alone (not shown), double-stained for IgE and J chain (Fig. 5, *C*), or double-stained for IgE and tryptase (Fig. 5, *D*). As expected, IgE+ cells were significantly more prominent in the subepithelium of the allergic nasal mucosa than in that of the control tissues (17.4 and 3.9 cells/mm BM, respectively, $p < 0.001$) (Fig. 7). We then tried to determine which type of cells these IgE+ cells were. IgE+ cells failed to stain for J chain even in the nasal mucosa of allergic patients (Fig. 5, *C*). On the other hand, double-staining for IgE and tryptase revealed that more than 90% of IgE+ cells costained for tryptase in the allergic subjects (Fig. 5, *D*). The proportion of mast cells that costained for IgE was significantly higher in the allergy group than in the control group (66% and 15%, respectively, $p < 0.001$; Fig. 7).

DISCUSSION

Classically, allergic rhinitis is caused by a type I allergic reaction. Antigens bind to IgE antibodies on mast cells and induce the release of mast cell mediators, which cause various allergic symptoms. However, as the functions of other inflammatory cells and those of their products are revealed, the possible role of these cells in allergic diseases becomes more intriguing. Although it may be relatively easy to examine the function of certain kinds of cells in *in vitro* models, the role of inflammatory cells at the local site of allergic diseases remains unclear. Recently, immunohistochemical determination of cell types as defined by cell-surface markers in specific tissues has become possible. For these studies, frozen (cryostatic) sections are commonly used because of the loss of the antigenicity of cell-surface markers during standard tissue preparation involving fixation in formalin. However, in cryostatic sections, spaces in the stroma are often enlarged, which makes cellular quantification inaccurate. We used a freeze-dried

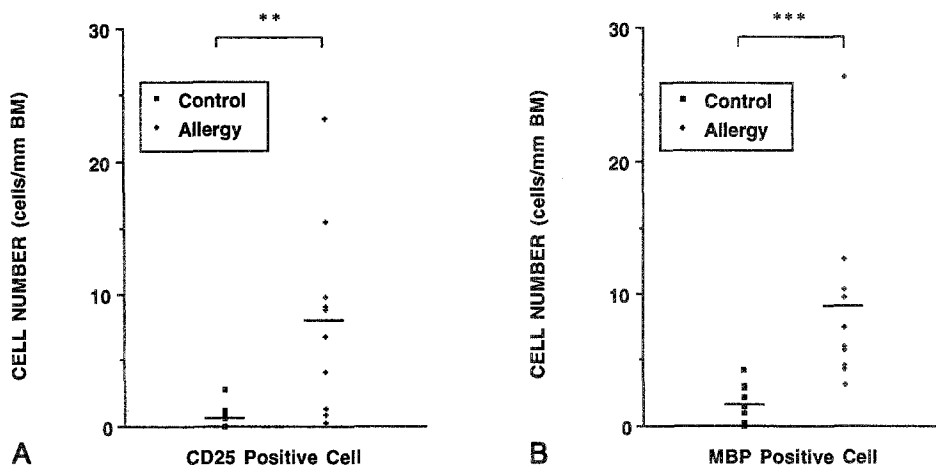


FIG. 4. Number of CD25+ cells and eosinophils in the nasal mucosa. Positively stained cells were counted in the superficial 200 μ m below the BM in the lamina propria and were divided by the length of the BM. **A**, CD25+ cells. **B**, MBP+ cells. Each point represents the result from an individual patient's tissue. Cross bars represent the mean. Asterisks indicate significant differences between the allergy group and the control group (Mann-Whitney U test). ** $p < 0.01$; *** $p < 0.001$.

paraffin-embedding technique to obtain both preserved antigenicity and better tissue structure than that obtainable with cryostatic sections.¹⁶

In initial studies we examined surgically removed inferior nasal turbinates obtained from patients with sleep apnea and found that the cells were distributed uniformly throughout the anterior 80% of the turbinate (data not shown). The anterior portion of the turbinate is subjected to maximal airflow and therefore is the probable site of impact for many allergens and potentially infectious substances. Therefore we believe that examination of the nature of the inflammatory cells of the anterior portion of inferior turbinates should provide insights into the cellular response of the nasal and airway mucosa to aeroallergens.

In agreement with results of previous studies,^{14, 15} T cells were the most abundant inflammatory cells noted in the nasal mucosa, and the number of CD4+ cells exceeded that of CD8+ cells. Variations of these cell numbers among individuals were so large that no significant differences between the allergy group and the control group were noted in the number of T cells, B cells, or macrophages.

Contrary to the report by Bentley et al.,¹⁵ we observed an increase in CD25+ cells in the allergic group as compared with the control group. Our findings are in agreement with those of Varney et al.²¹ who reported an increase in CD25+ cells after topical allergen challenge. The intensity of allergen exposure may be responsible for the differences between the studies. CD25, an antigen on the

α -chain of the IL-2 receptor, has been reported to appear after the activation of not only T cells²² but also B cells,²³ natural killer-cells,²⁴ and monocytes.²⁵ However, the CD25+ cells observed in this study were located primarily in the superficial submucosa, a distribution most consistent with T cells. These data suggest that nearly 10% of CD3+ T cells in biopsy specimens from patients with symptomatic allergic rhinitis are activated. Recently, the concept of categorizing helper T cells into two subtypes on the basis of cytokine secretion patterns, initially described in murine models,²⁶ has become popular in human models. T_{H1} type cells produce IL-2 and γ -interferon, but not IL-4 and IL-5, whereas T_{H2} type cells produce IL-4 and IL-5, but not IL-2 and γ -interferon.²⁶ Del Prete et al.²⁷ separated T_{H1} type and T_{H2} type clones from human peripheral blood lymphocytes. Durham et al.²⁸ demonstrated T_{H2} type cytokine expression by in situ hybridization study of nasal mucosa after allergen provocation. Thus it is possible that the CD25-bearing cells observed in the biopsy specimens from allergic patients were lymphocytes bearing the T_{H2} phenotype.

The number of eosinophils was also higher in the patients with allergy than in the normal subjects. It is likely that some of these eosinophils are activated, because Bentley et al.¹⁵ reported the increased detection of cells positive for the cleaved form of eosinophil cationic protein in allergic subjects. Unlike the role of the lymphocyte, it is generally accepted that selective recruitment of eosinophils is important in the pathogenesis of

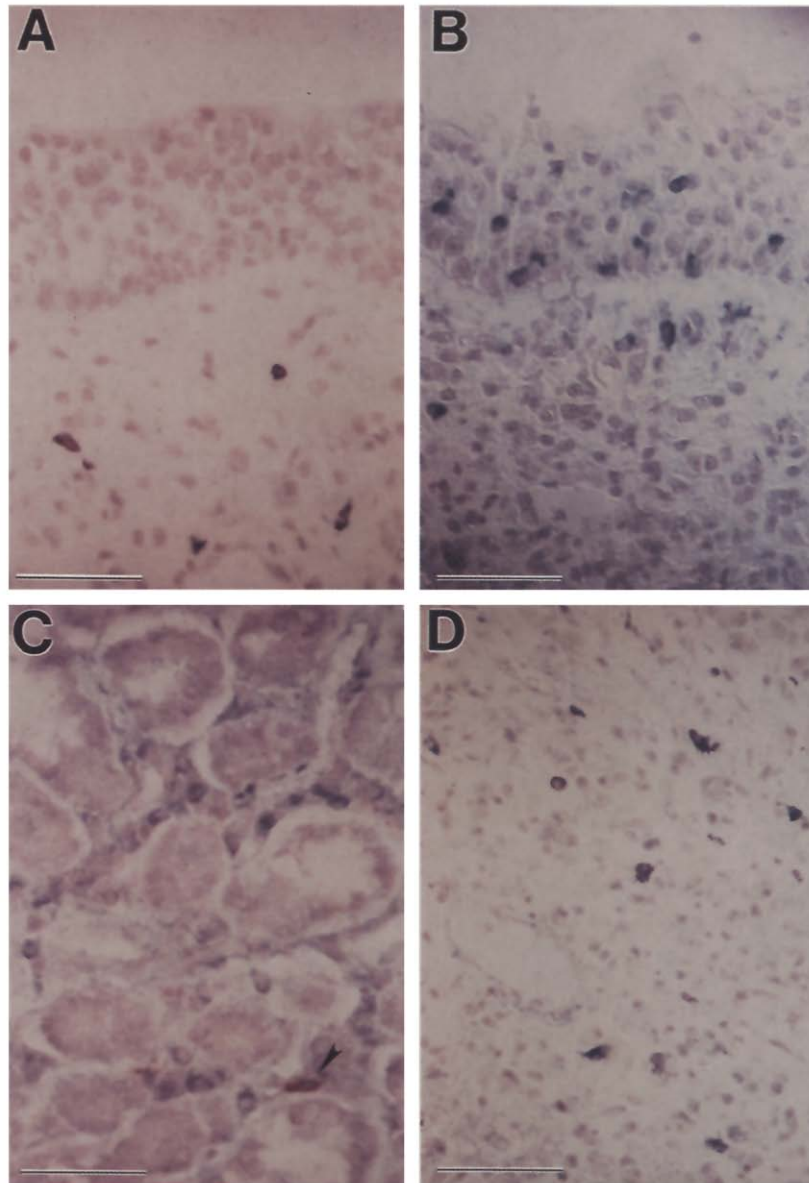


FIG. 5. Staining of nasal mucosa for mast cells. **A and B,** Sections of Carnoy's fixed nasal biopsy tissue were double-stained for chymase (brown) and tryptase (blue); biopsy specimens from a control subject (**A**) and from a patient with allergic rhinitis (**B**). **C,** Biopsy specimen from a patient with allergic rhinitis stained for IgE (brown; arrowhead) and for J chain (blue). **D,** Biopsy specimen from a patient with allergic rhinitis stained for IgE (brown) and for tryptase (blue). Scale bars indicate approximately 50 μ m.

allergic rhinitis. The migration of eosinophils may be the result of T-cell or granulocyte products such as IL-5,²⁹ leukotriene B₄,³⁰ platelet-activating factor,³¹ and leukotriene E₄,³² but it is also possible that some cytokines such as IL-3,³³ IL-5,¹⁰ and granulocyte-macrophage colony-stimulating factor¹¹ enhance the survival and the activation of eosinophils.

There were no differences in total mast cell numbers between the allergic rhinitis group and

the normal control group. Bentley et al.¹⁵ found more intraepithelial mast cells in allergic patients than in the normal subjects. Our data were consistent with their findings; however, our differences were not statistically significant. In contrast, although the former study did not find any differences in mast cell subsets between patients with allergic rhinitis and normal subjects, we detected a slight but significant difference in subepithelial MC_T number. Because intraepithelial mast cells

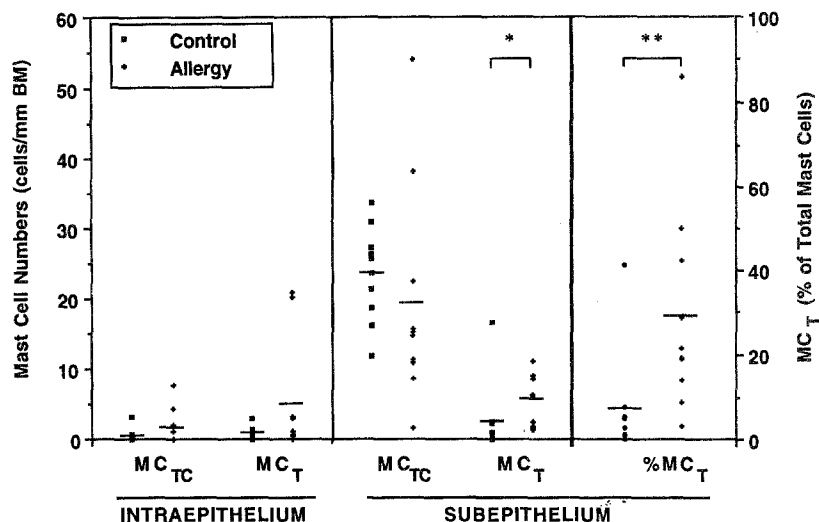


FIG. 6. Number of mast cells in the nasal mucosa. Positively stained cells were counted in the epithelium, as well as in the superficial 200 μ m below the BM in the lamina propria and were divided by the length of the BM. The numbers of MC_{TC} and MC_T in the epithelium are shown on the *left*, and those in the subepithelium are shown in the *middle* (both expressed as cell numbers per millimeter of BM). The relative proportion of MC_T to the total mast cells ($MC_{TC} + MC_T$) in the subepithelium are shown on the *right*. Each point represents the result from an individual patient's tissue. Cross bars represent the mean. Asterisks indicate significant differences between the allergy group and the control group (Mann-Whitney U test). * $p < 0.05$; ** $p < 0.01$.

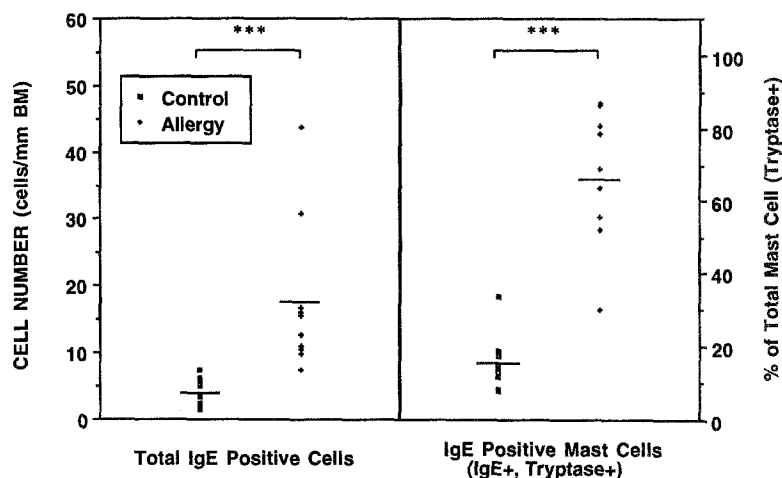


FIG. 7. Number of IgE+ cells in the nasal mucosa. Positively stained cells were counted in the superficial 200 μ m below the BM in the lamina propria and were divided by the length of the BM. The number of total IgE+ cells are shown on the *left*, and the number of IgE+ mast cells (both IgE+ and tryptase+) are shown on the *right*. Each point represents the result from an individual patient's tissue. Cross bars represent the mean. Asterisks indicate significant differences between the allergy group and the control group (Mann-Whitney U test). *** $p < 0.001$.

(mostly MC_T ^{15,16}) are increased in seasonal allergic rhinitis¹⁵ and migrate to the epithelium from the subepithelium,³⁴ it is not surprising that MC_T were increased in the subepithelium. When our data are combined with that of others,^{15,34} it seems that MC_T in the nasal mucosa of patients with allergic rhinitis are increased in number and mi-

grate into the epithelium, although it is sometimes difficult to obtain statistical significance in clinical studies. The proliferation of MC_T is known to be T cell-dependent, and it has been reported that patients with immunodeficiency lack MC_T in the intestinal mucosa.³⁵ However, although T-cell products may be necessary, they may not be suffi-

cient for MC_T differentiation, because the numbers of CD25+ cells and those of MC_T did not correlate in our study (data not shown). It is also possible that factors released from activated T cells or mast cells (such as IL-3) influenced the proportion of mast cell subtypes. The physiologic roles of MC_T and MC_{TC} are unclear. However, mucosal type mast cell (MC_T) activation is more specific for IgE because these cells, as opposed to MC_{TC}, are refractory to activation by non-IgE-mediated stimuli such as C5a³⁶ or N-formyl-methionyl-leucyl-phenylalanine.³⁷

As expected, more IgE+ cells were seen in the allergic group than in the control group, which is in agreement with results of previous studies.^{14, 38} Interestingly, most of the IgE+ cells also stained for mast cell tryptase, and IgE+, tryptase-negative cells were rare even in patients with allergic rhinitis. We could not double-stain for IgE and J chain. These observations suggest that IgE is seen only on mast cells and not B cells and therefore that IgE may not be produced locally in the nasal mucosa. Indeed, Ganzer and Bachert³⁹ were able to demonstrate IgE and CD38 (stains plasma cells and some T cells) double-positive cells in the tonsils but not in the nasal mucosa of subjects with seasonal allergies. It is possible that the low sensitivity of IgE staining prevented the detection of IgE+ plasma cells in the nasal mucosa. However, B cells in the nasal mucosa migrate to periglandular regions when they produce immunoglobulins,^{16, 40} whereas mast cells are distributed throughout the tissue. If IgE production occurred locally in the nasal mucosa, the IgE-producing plasma cells would have to secrete large amounts of IgE into the interstitium to be taken up by mast cells. If so, one would anticipate that IgE-secreting plasma cells would stain positively in the interstitium. There were no such cells stained, even after overdeveloping the interstitium (not shown). It is also possible that staining of one antigen (IgE) prevented staining of the other antigen (J chain) because the two antigens are located in a single cell. However, IgA and J chain were readily double-stained in the same tissue (not shown). Our data suggest that most of the IgE+ cells in the nasal mucosa are mast cells and are consistent with the hypothesis that the lymph nodes are the primary site of IgE production. Thus it is conceivable that mast cell progenitors migrating into the nasal mucosa pass through lymph nodes where they bind IgE. If this is true, then it is unlikely that resident mast cells of the nasal mucosa are capable of acquiring IgE to new antigens. Moreover, it may be

important to assess the cytokine profiles of T cells in the lymph nodes in order to test the relationship of T_{H2} cells, IgE production, and allergic disease.

In summary, symptomatic atopic subjects who underwent biopsy during their allergy season had similar numbers of T cells, CD4+ and CD8+ cells, B cells, and macrophages in their nasal mucosa as compared with nonatopic control subjects. Activation of T cells, as assessed by the presence of CD25+ cells in the superficial submucosa, was increased in allergic subjects, however. This increase occurred concomitantly with an increase in eosinophils and in mucosal type (MC_T) mast cells in the atopic group's nasal mucosa. The proportion of mast cells staining for IgE was also higher in the atopic group, and non-mast cells bearing IgE were rare in both groups. These data suggest that qualitative, as opposed to quantitative, changes in lymphocytes and mast cells occur during ongoing antigen exposure and that IgE may be produced in local lymphoid tissue rather than in the nasal mucosa.

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