

The Histology of Allergic Rhinitis and Its Comparison to Cellular Changes in Nasal Lavage

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To better understand how changes in cells in nasal secretions reflect changes in the nasal mucosa occurring during allergic reactions, we obtained nasal lavage and biopsy specimens from normal ($n = 11$) asymptomatic, seasonally allergic ($n = 18$), and perennially allergic ($n = 18$) subjects. Initial baseline lavages showed that perennially, and seasonally allergic subjects, out of their allergy seasons, had significantly higher numbers of eosinophils ($p < 0.01$) and neutrophils ($p < 0.01$) and total cell counts ($p < 0.05$) than normal subjects. Biopsy results showed that at baseline, seasonally allergic subjects had thicker mucosa ($p < 0.01$), greater numbers of intraepithelial mononuclear cells and total cells ($p < 0.01$), and greater numbers of subepithelial neutrophils ($p < 0.001$) than perennially allergic subjects. Twenty-four hours after antigen provocation, nasal lavage of allergic subjects showed an increase in the number of eosinophils ($p < 0.05$). Seasonally allergic subjects also had significant increases in numbers of intraepithelial mononuclear cells ($p < 0.05$) and total cells ($p < 0.01$), and in subepithelial eosinophils ($p < 0.001$) and mononuclear cells ($p < 0.05$), which were localized to the side challenged. Despite an influx in eosinophils, the epithelial layer was not changed from baseline. The data provide evidence that nasal secretions and the nasal mucosa represent two distinct cellular compartments. **Lim MC, Taylor RM, Naclerio RM. The histology of allergic rhinitis and its comparison to cellular changes in nasal lavage. *Am J Respir Crit Care Med* 1995; 151:136-44.**

The upper airway provides an unparalleled opportunity to study the cellular changes in the mucosa occurring during an allergic reaction. To date, studies have examined cellular changes in nasal secretions, epithelial scrapings or imprints, and biopsies following either antigen provocation or natural exposure. In provocation studies, Bascom and colleagues documented a small but significant increase in eosinophils within 1 h of antigen challenge, but the dramatic increase occurred 7 to 11 h later (3, 4). Pipkorn and colleagues showed a significant increase in eosinophils after allergen challenge, which was maintained throughout 7 d of challenge (2). Pelikan, on the other hand, reported that in the late reaction, eosinophils increased immediately after allergen challenge and decreased during the appearance of the late nasal response (5). In nasal biopsy specimens, Connell showed influx of eosinophils, histiocytes, and foam cells in nasal biopsies only on the side challenged (7). Wihl and Mygind, also using biopsy specimens, demonstrated a significant increase in perivascular eosinophils but no change in mast-cell density 30 min after challenge (8). Using immunohistochemical staining of nasal biopsies specimens, other investigators have described changes in lymphocytes and Langerhan cells hours after challenge (9-15).

Similar changes have been observed in studies performed after seasonal antigen exposure. In nasal secretions, Pipkorn and

colleagues found a seasonal increase in the number of eosinophils of persons with birch pollen rhinitis, with a close correlation between the number of eosinophils, nasal symptoms, and pollen count (1). Utilizing nasal imprints, which evaluate cells in secretions and in the superficial epithelial layer, Pipkorn also showed an increase in mast-cell density after 4 to 5 d of natural pollen exposure (1). Locewicz and colleagues obtained nasal biopsies from 22 patients with seasonal allergic rhinitis to grass pollen before and after the season. Exposure to allergen was accompanied by a significant increase in the density of eosinophils and mast cells (6). From these studies it is clear that changes occur in the cellular composition of the nasal mucosa following an allergic reaction.

In this study we sought to: (1) simultaneously study the leukocyte composition of nasal secretions, epithelium, and submucosa at baseline in normal subjects, and asymptomatic persons with seasonal allergies; (2) determine the effect of antigen provocation on cells in nasal secretions and biopsies in subjects with seasonal allergic rhinitis; (3) determine whether leukocyte infiltration occurs only at the site of antigen challenge; (4) describe the histology of the mucosal epithelium and goblet cells, subepithelial collagen, and mucoserous glands in allergic and nonallergic subjects before and after antigen challenge; and (5) compare the histology of persons with seasonal allergic rhinitis after challenge with the histology of persons with symptomatic perennial allergic rhinitis.

METHODS

Subjects

We performed a series of studies to address our aims. First, we challenged

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seasonal allergic and nonallergic subjects with antigen and measured their response at the site of challenge. Next, we challenged allergic subjects and measured their response at the site of the challenge and in the opposite nostril. Finally we obtained biopsies from symptomatic perennially allergic subjects. In total, nasal biopsies were performed on 11 normal subjects (three men and eight women, mean age 26.5 yr), 18 seasonally allergic subjects (nine men and nine women, mean age 40 yr), and 18 perennially allergic subjects (11 men and seven women, mean age 31 yr).

Each subject was skin-tested with diluent control and a panel of antigens, including short ragweed, English plantain, timothy grass, orchard grass, white oak tree, maple tree, molds, house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), and dog and cat dander (allergenic extract and sterile albumin saline with phenol; Hollister Stier, Inc., Spokane, WA). Normal subjects had no history of allergic symptoms and negative intradermal skin tests to ≤ 100 protein nitrogen units (PNU) of the antigen. Seasonally allergic subjects had an appropriate history for grass or ragweed pollenosis and a positive skin puncture test. All seasonally allergic subjects were asymptomatic and were studied at a time remote from their allergy season. Perennially allergic subjects had rhinitic symptoms throughout the year and showed positive reactions to house-dust-mite antigen and to one or more other allergens. All subjects were in good health and were not on medications for at least 6 wk prior to biopsy. The protocol received institutional review board approval, and each participant gave informed consent prior to entering the study.

Study Design

In subjects not undergoing nasal challenge, nasal lavages samples were obtained immediately before nasal biopsy. In subjects undergoing a nasal challenge with antigen, a nasal lavage sample was obtained before the antigen challenge and 24 h later, immediately before nasal biopsy.

Nasal Lavage for Cells

Five milliliters of lactated Ringer's solution (Kendall McGaw Laboratories Inc., Irvine, CA) at 37° C was instilled into each nostril. This lavage technique covers both nostrils and the nasopharynx. The exact site of origin of cells obtained by lavage cannot be determined. After 10 s the subjects flexed their neck and expelled the solution into a plastic basin. The fluid was then transferred to 15-ml polypropylene centrifuge tubes with screw caps (VWR Scientific, Philadelphia, PA) and shaken vigorously. The total number of cells in the expelled lavage fluid was counted with a Neubauer hemacytometer (Reichert Scientific Instruments, Buffalo, NY). At least 100 cells, or the number of cells in nine large squares, were counted. Only nucleated cells and epithelial cells were counted; rare red blood cells were excluded. Next, the collected nasal lavages were centrifuged at $1500 \times g$ for 15 min and the supernatant was separated from the pellet. The pellet was then resuspended in enough lactated Ringer's solution to provide a final suspension of 100,000 cells/200 μ l of lactated Ringer's solution. Two hundred microliters of the final suspension were then cytospun on slides at 600 rpm for 6 min (Shandon Cytospin 2, Pittsburgh, PA) and allowed to air dry. Slides were stained with the modified Wright technique (Diff-Quik® Stain set, Barter Health-Care Corp., Miami, FL), and the percentage of eosinophils, neutrophils, and mononuclear cells was determined. The total cell count for an individual cell type was determined by the product of the total cell count and the fraction of that type of cell in the differential count.

Antigen Challenge

Antigen challenges were performed on both the normal subjects and those with seasonal allergic rhinitis. An 8 mm disk (punched out Shandon filter cards; Shandon, Pittsburgh, PA) with 50 μ l of the relevant antigen extract (333 PNU grass or ragweed) was applied to the anterior tip of the inferior turbinate for 1 minute. Sneezes were counted and nasal secretions were collected 30 s later by applying preweighed disks to the site of challenge for 30 s.

Nasal Biopsy

Local anesthesia and vasoconstriction of the anterior tip of the mucosa of the inferior turbinate were obtained by topical application of 2% pontocaine and oxymetazoline (Nostrilla®, Boehringer Ingelheim Pharmaceu-

ticals, Inc., Ridgefield, CT), followed by injection of 1% lidocaine with 1:100,000 epinephrine (Abbott Laboratories, North Chicago, IL). Fifteen minutes later, a biopsy of the mucosa of the inferior turbinate was done with an adenoid punch forceps. The anterior tip was chosen because of its location within the airstream and its accessibility, which allowed us to obtain a large specimen without a crushing artifact. Biopsy specimens measuring about 5×3 mm in diameter were prepared for light microscopy. Each sample was fixed in 10% buffered formalin (Richard-Allan, Richland, MI), progressively dehydrated in an Autotechnicon Model 2A (Miles Inc., Tarrytown, NY) tissue processor, cleared in HistoClear (National Diagnostics, Monville, NJ), and embedded in paraffin (Paraplast Tissue Embedding Medium, Oxford Labware, St. Louis, MO) under vacuum. Each specimen was sectioned on a Leitz 1512 microtome at 5 μ m thickness, placed on a glass slide, and stained as follows: routine Harris hematoxylin and eosin (H&E) to identify and quantitate leukocytes; periodic acid-Schiff with diastase digestion (PASD) to quantitate goblet cells and mucoserous glands; and Masson trichrome (MT) to identify the subepithelial collagen and measure its thickness. Examination of the glass slides was performed with an American Optical double-headed light microscope. One eyepiece of the microscope contained an American Optical linear micrometer, while the other contained an American Optical 1 cm² grid reticule divided into 100 squares to quantitate cells and tissue per square millimeter. The linear micrometer contains 100 divisions at 100 and 400 $\times$ magnification, each division having a conversion factor of 0.0098 mm and 0.0025 mm, respectively. The micrometer was placed along the linear length of the mucosal epithelium to quantify the density of goblet cells and intraepithelial inflammatory cells per linear millimeter. It was also placed vertically at intervals to measure the average thickness of mucosal epithelium and subepithelial collagen. Under 400 $\times$ magnification, 100 squares of the 1-cm² grid reticule measure 0.0625 mm² of tissue. We then proceeded to quantify the inflammatory cells per square millimeter of lamina propria by counting eight squares in the central portion of the biopsy and then multiplying by a factor of two. Under 100 $\times$ magnification, 100 squares measure essentially 1 mm² of tissue. We then proceeded to quantify the mean density of mucoserous glands in the subepithelial area per square millimeter of tissue under 400 $\times$ magnification, except for the quantification of the seromucous glands, which were interpreted under 100 $\times$ magnification. Slides were interpreted by two independent observers unaware of the challenge conditions.

Because of poor adhesion of the epithelium, there was insufficient epithelium to interpret for one normal subject, one seasonally allergic subject without antigen provocation, and three seasonally allergic subjects after antigen provocation. The slides of one seasonally allergic subject with and without antigen provocation utilizing the PASD stain could not be interpreted.

Statistical Analysis

Nonparametric statistical methods were employed. Kruskal-Wallis analysis of variance (ANOVA) was utilized to evaluate significant differences among groups. Further analysis was made using Wilcoxon's matched-pairs signed-rank test. For nonpaired data, analysis was made using the Mann-Whitney U test. To determine correlations, we used Spearman's rank correlation coefficient test. Two-tailed p values < 0.05 were considered significant. In the text, the data are expressed as mean $\pm$ SEM to show variance. Individual data and the medians are presented in the figures.

Histologic Definitions

The mucosa of the anterior tip of the inferior nasal turbinate can display three types of epithelium: (1) pseudostratified ciliated columnar respiratory epithelium; (2) intermediate epithelium of mixed stratified cuboidal to cuboidal-squamous type; and (3) stratified squamous "metaplastic" epithelium (16). Our study tissue displayed all three forms of epithelial change.

RESULTS

Symptoms

Following antigen provocation, the allergic subjects had significantly more sneezes (0 versus 11.4 ± 2.2 , $p < 0.01$) and a significant increase in secretion weight (8.6 ± 1.5 versus 45.1 ± 3.9 ,

$p < 0.01$) from baseline. The allergic subjects also had significantly more sneezes (11.4 ± 2.2 versus 0, $p < 0.001$) and secretion weights after antigen challenges (45.1 ± 3.9 versus 4.4 ± 1.2 , $p < 0.001$) than the nonallergic group.

We present the cellular data according to the compartments in which the cells were collected, comprising: surface secretions, epithelium, and lamina propria. Nasal lavages provided information about the secretions, whereas biopsies provided information about the epithelium and lamina propria. It is important to note that the seasonally allergic group and normal subjects underwent allergen provocation, whereas the perennially allergic subjects were not challenged.

Lavage

The total numbers (mean $\pm$ SEM) of cells obtained by lavage at baseline from seasonally allergic subjects (asymptomatic and out of season), perennially allergic subjects, and normal subjects were $8.2 \pm 3.9 \times 10^5$, $2.2 \pm 0.9 \times 10^5$ and $0.5 \pm 0.3 \times 10^5$, respectively. The predominant leukocyte in all subjects was the neutrophil ($73.4 \pm 9.9\%$). Baseline lavage specimens from the perennially allergic subjects contained significantly more eosinophils ($p < 0.01$), neutrophils ($p < 0.01$), and total cells ($p < 0.05$) than those of the normal subjects. Likewise, seasonally allergic subjects had significantly more neutrophils ($p < 0.001$) and total cells ($p < 0.001$) than did normal subjects. In addition, the seasonally allergic subjects had significantly more neutrophils ($p < 0.05$) and total cells ($p < 0.05$) than the perennially allergic subjects (Figure 1).

Seasonally allergic subjects showed a significant increase in

the number of lavage eosinophils ($0.32 \pm 0.2 \times 10^5$ to $1.2 \pm 0.8 \times 10^5$, $p < 0.05$) 24 h after antigen challenge. There were no significant changes noted in the normal group of subjects after antigen challenge (Figure 2). The lavages from the seasonally allergic subjects following challenge had significantly higher numbers of eosinophils ($p < 0.001$), neutrophils ($p < 0.001$), and total cell counts ($p < 0.001$) than those of normal subjects.

Biopsy

Epithelium. Three normal subjects had areas of pseudostratified respiratory epithelium that gradually merged with areas of stratified squamous epithelium. Five subjects had only stratified squamous epithelium, and two subjects had stratified squamous epithelium with focal thickening. Twelve of 17 seasonally allergic subjects before challenge and 12 of 16 after antigen challenge had stratified squamous epithelium. The remaining five prechallenge and four postchallenge subjects showed pseudostratified respiratory epithelium with focal areas of intermediate change. Perennially allergic subjects had a more varied appearance: one subject exhibited only pseudostratified respiratory epithelium, six had stratified squamous epithelium, three had stratified squamous epithelium, and seven had stratified squamous metaplastic epithelium.

The thickness of the epithelium in the normal group of subjects was 0.04 ± 0.01 mm. Unchallenged seasonally allergic subjects had thicker epithelium (0.07 ± 0.01 mm, $p < 0.01$) (Figure 3B) than did the normal subjects. After antigen challenge, seasonally allergic subjects maintained a thicker epithelium $0.07 \pm$

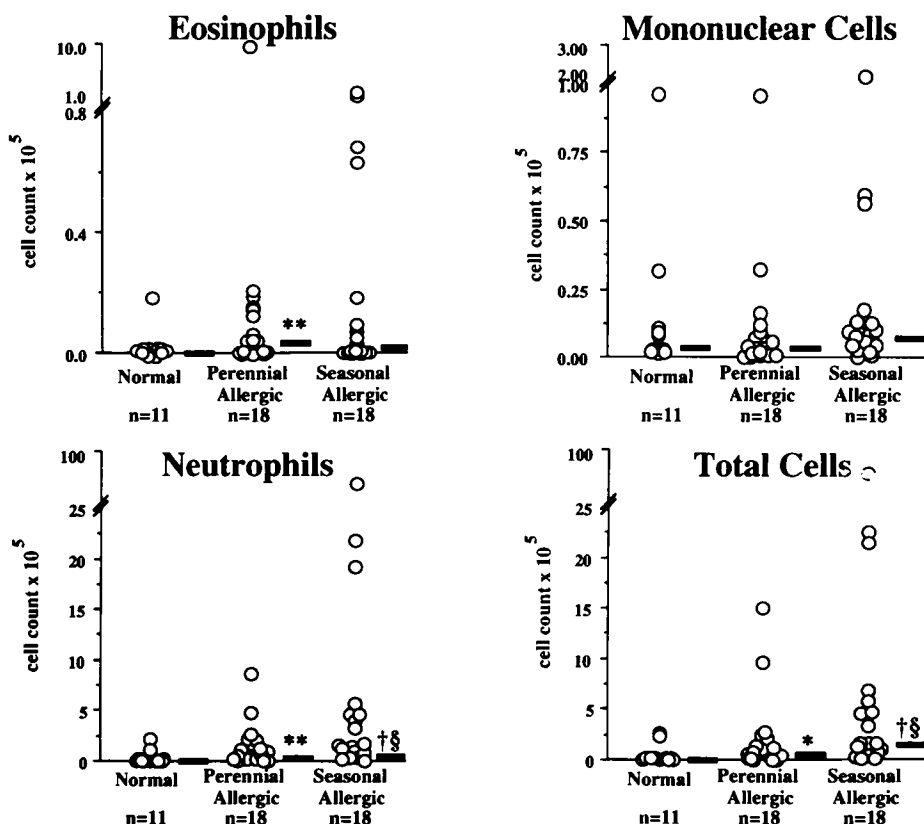


Figure 1. Baseline lavages from normal, perennially allergic, and seasonally allergic rhinitic subjects. Medians are depicted by the dark horizontal bars. ** $p < 0.01$ and * $p < 0.05$ = comparison between perennially allergic subjects and normal subjects. † $p < 0.05$ comparison between seasonally allergic subjects and perennially allergic subjects. § $p < 0.001$ comparison between seasonally allergic subjects and normal subjects.

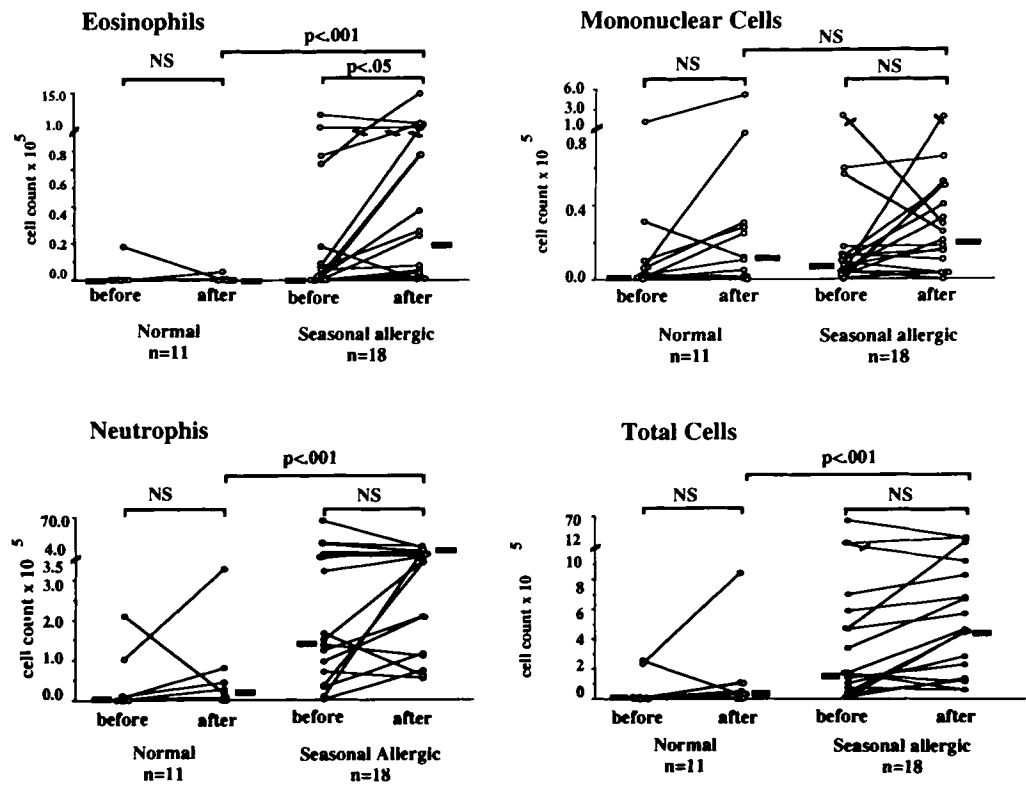


Figure 2. Cells in nasal lavages of normal and seasonally allergic rhinitic subjects before and after antigen provocation. NS = nonsignificant.

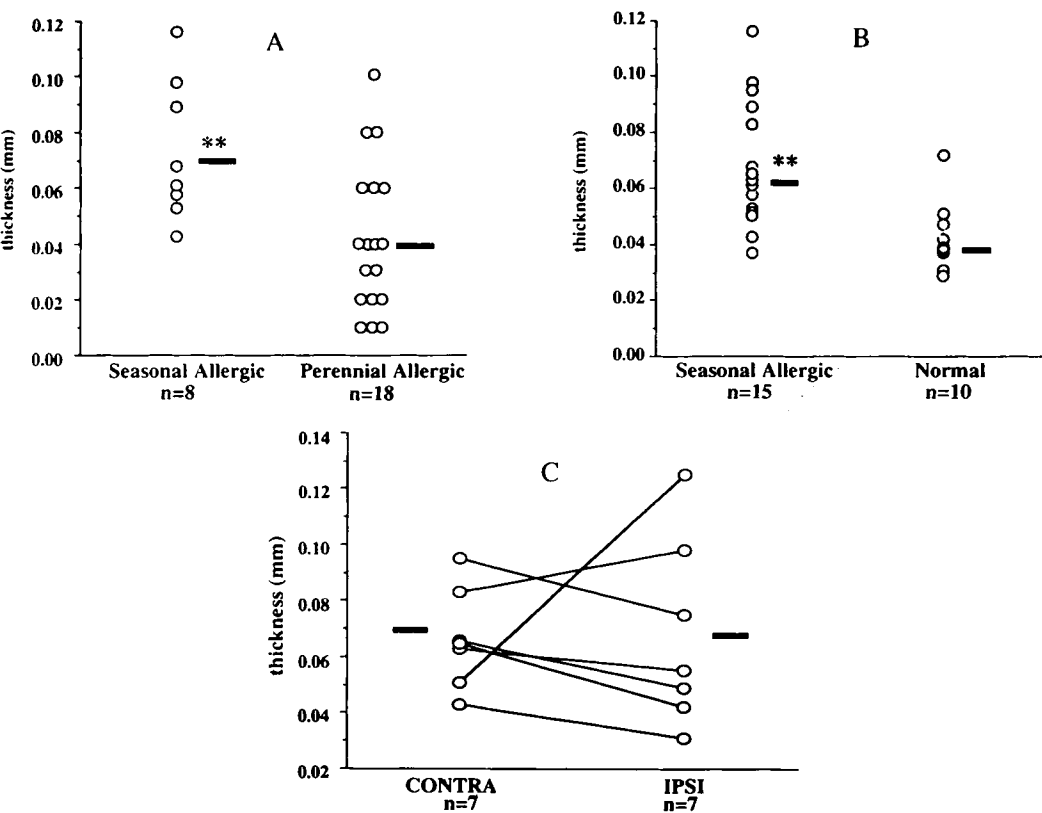


Figure 3. Epithelial thickness. (A) Seasonally and perennially allergic rhinitic subjects. **p < 0.01 compared with perennially allergic subjects. (B) Seasonally allergic rhinitic and normal subjects after antigen provocation. **p < 0.01 compared with normal subjects. (C) Contralateral and ipsilateral sides of the nose after ipsilateral antigen provocation. Median is depicted by the dark horizontal bar.

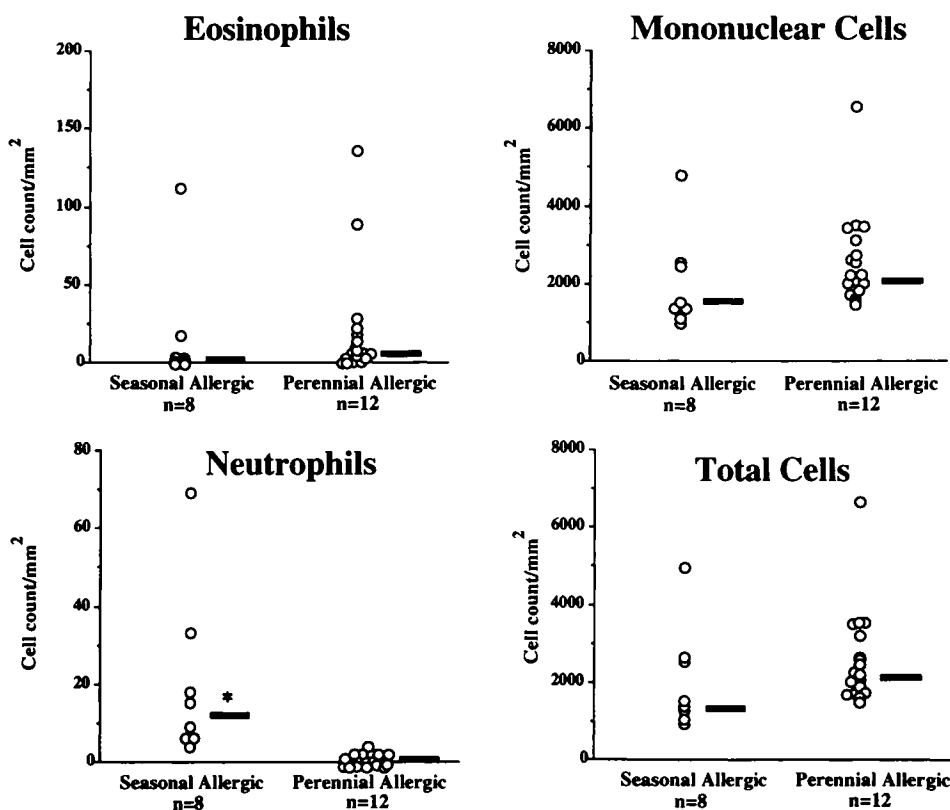


Figure 4. Nasal biopsies of unchallenged nasal mucosa were obtained from seasonal and perennial allergic rhinitic subjects. The numbers of total cells, eosinophils, neutrophils, and mononuclear cells were counted per square millimeter of lamina propria. Median is depicted by the dark horizontal bars. * $p < 0.001$.

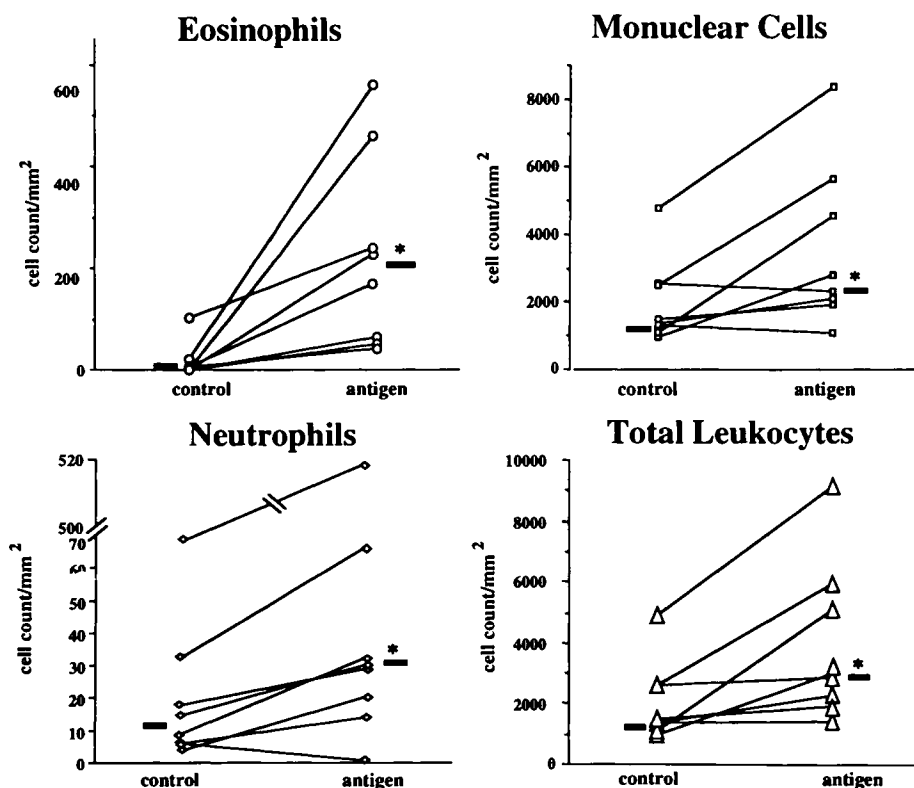


Figure 5. Nasal biopsies of seasonally allergic subjects 24 h after challenge with antigen, compared with the same individuals challenged with a control solution on another day. Median is depicted by the dark horizontal bars. $n = 8$; * $p < 0.05$.

0.01 mm ($p < 0.01$) than did the normal subjects. The ipsilateral epithelial thickness of the seasonally allergic group of rhinitis patients obtained 24 h after antigen provocation did not differ from that on the unchallenged contralateral side or from baseline (Figure 3C). The unchallenged perennially allergic subjects (0.04 ± 0.01 mm) (Figure 3A) had significantly thinner epithelium than the seasonally allergic subjects ($p < 0.01$).

Subepithelial inflammatory cells. In all groups, the predominant leukocyte in the tissue was the mononuclear cell. The total number of cells for unchallenged seasonally allergic subjects was $2.1 \pm 0.5 \times 10^3$ cells/mm², which was similar to the value of $2.6 \pm 0.3 \times 10^3$ cells/mm² for perennially allergic subjects. Seasonally allergic subjects had significantly higher numbers of neutrophils ($p < 0.001$) than perennially allergic subjects (Figure 4).

We first compared the response of seasonally allergic subjects biopsied at baseline with their results 24 h after antigen challenge. In seasonally allergic subjects the total number of cells increased from $2.1 \pm 0.5 \times 10^3$ before challenge to $3.8 \pm 0.5 \times 10^3$ cells/mm² ($p < 0.001$) after challenge. Seasonally allergic subjects showed significant increases in tissue eosinophils ($0.01 \pm 0.006 \times 10^3$ versus $0.2 \pm 0.04 \times 10^3$ cells/mm², $p < 0.05$) and mononuclear cells ($2 \pm 0.2 \times 10^3$ versus $3.6 \pm 0.4 \times 10^3$ cells/mm², $p < 0.001$) after antigen provocation (Figure 5).

In another group of seasonally allergic subjects, we challenged one side with antigen and biopsied both sides of the nose 24 h later. Ipsilateral nasal challenge showed a significantly greater number of eosinophils ($0.13 \pm 0.03 \times 10^3$ versus $0.01 \pm 0.003 \times 10^3$, $p < 0.01$) and mononuclear cells ($3.5 \pm 0.5 \times 10^3$ versus $2 \pm 0.2 \times 10^3$, $p < 0.05$), and a greater total cell count ($3.7 \pm 0.5 \times 10^3$ versus $2.2 \pm 0.3 \times 10^3$, $p < 0.01$) than was found on the contralateral side of the nose (Figure 6). The ipsilateral increases af-

ter antigen challenge in seasonally allergic subjects were significantly greater than the response of nonallergic subjects challenged with antigen (Figure 7) with regard to eosinophils ($0.05 \pm 0.04 \times 10^3$ versus $0.2 \pm 0.04 \times 10^3$ cells/mm², $p < 0.001$), mononuclear cells ($2.1 \pm 0.2 \times 10^3$ versus $3.6 \pm 0.4 \times 10^3$ cells/mm², $p < 0.05$), and total cells ($2.3 \pm 0.3 \times 10^3$ versus $3.8 \pm 0.5 \times 10^3$ cells/mm², $p < 0.05$).

Ipsilateral nasal challenge was also compared with the findings in unchallenged perennially allergic subjects, and showed significantly higher numbers of eosinophils ($0.2 \pm 0.04 \times 10^3$ versus $0.02 \pm 0.01 \times 10^3$ cells/mm², $p < 0.001$), neutrophils ($0.1 \pm 0.04 \times 10^3$ versus $0.002 \pm 0.001 \times 10^3$ cells/mm², $p < 0.001$), and total cells ($3.8 \pm 0.5 \times 10^3$ versus $2.6 \pm 0.3 \times 10^3$ cells/mm², $p < 0.05$).

The data for intraepithelial inflammatory cells, goblet cells, subepithelial collagen, and mucoserous glands are presented in Table 1. The only differences found for these variables among the groups were increased epithelial total values in the allergic subjects after challenge as compared with normals, and increased mononuclear cells at baseline in seasonally allergic subjects as compared with perennially allergic subjects.

Correlation

There was no correlation between tissue eosinophilia and mucosal thickness in normal ($r = 0.20$), seasonally allergic ($r = 0.05$), or perennially allergic subjects ($r = 0.25$).

DISCUSSION

Since the allergic reaction is a dynamic process involving changing cell populations, most investigators have focused on study-

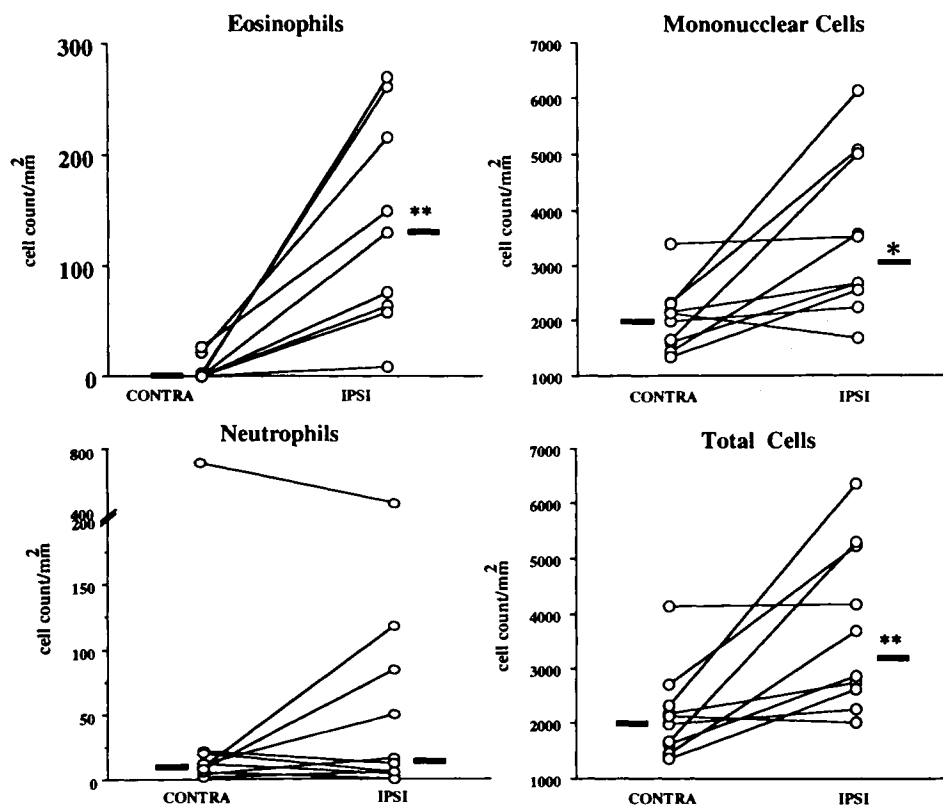


Figure 6. Nasal biopsies of the contralateral and ipsilateral sides of the nose 24 h after antigen challenge in seasonal allergic rhinitis. Median is depicted by the dark horizontal bars. $n = 10$; * $p < 0.05$; ** $p < 0.01$.

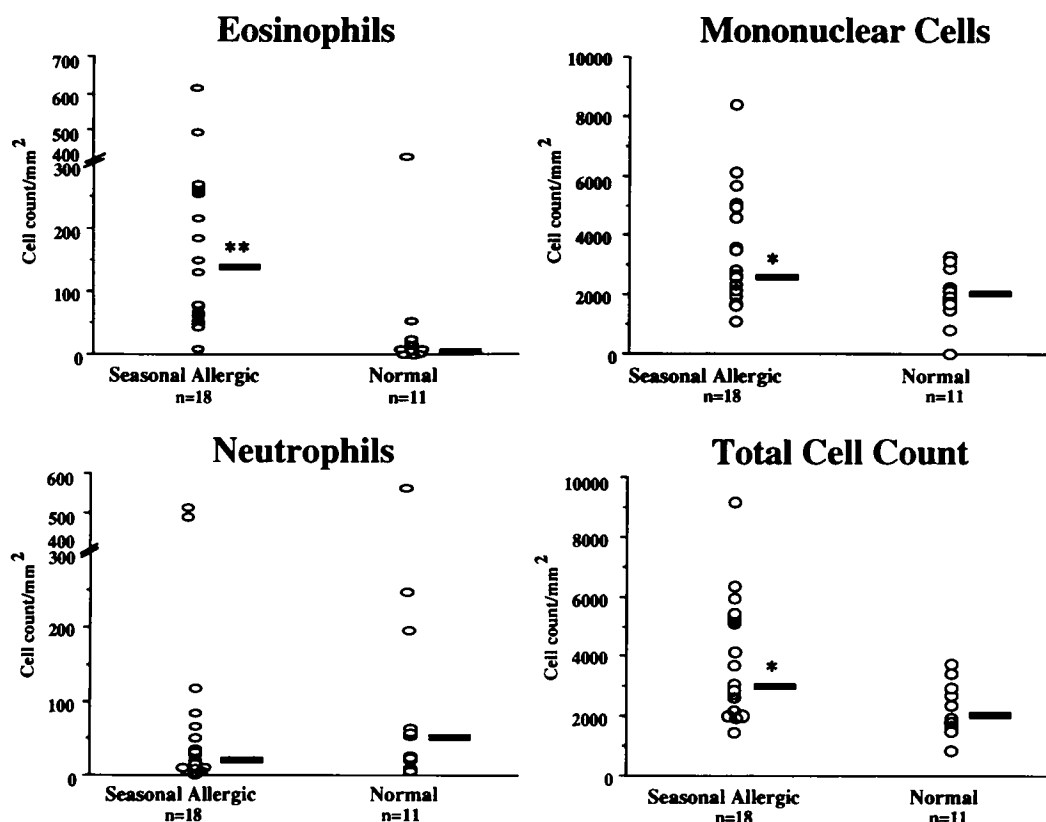


Figure 7. Comparison of nasal biopsies of normal and seasonally allergic rhinitic subjects 24 h after antigen challenge. The numbers of total cells, eosinophils, neutrophils, and mononuclear cells were counted per square millimeter of lamina propria. Median is depicted by the dark horizontal bars. * $p < 0.05$; ** $p < 0.001$.

ing cells on the epithelial surface, which can be sampled easily and frequently. This study was undertaken to simultaneously assess cells in nasal secretions and in the mucosa for changes after antigen provocation as well as for changes induced by chronic allergen exposure.

In the secretions, we showed that perennially allergic subjects had significantly more eosinophils, neutrophils, and total cells ($p < 0.001$) than normal subjects. Seasonally allergic subjects also had significantly more neutrophils and total cells than the normal and perennially allergic subjects. Since the seasonally allergic subjects were biopsied outside of their allergic season and were asymptomatic at the time of the study, we could not relate their neutrophil numbers to any clinical nasal disorder. Twenty-four hours after a localized antigen challenge, nasal lavages showed a dra-

matic increase in the number of eosinophils. The predominant leukocytic infiltrate in the nasal secretion, however, was the neutrophil. These results, achieved by stimulating a small area of the nose with an 8 mm disk, are consistent with those of previous studies in which large areas of the nose were challenged by an inhalation technique (3, 4, 17).

In nasal biopsy tissue, the predominant inflammatory cell in the epithelium and lamina propria was not the neutrophil but the mononuclear cell. These mononuclear cells had small, round, darkly staining nuclei and scanty cytoplasm, and could not consistently be classified by light microscopy as monocytes, lymphocytes, or macrophages. They are most likely lymphocytes, on the basis of immunohistochemical studies by others (10–13). After antigen challenge, seasonally allergic subjects showed a dramatic

TABLE 1
SELECTED HISTOLOGIC EVALUATIONS

	Normals	Allergic Patients Unchallenged	Allergic Patients Challenged	Perennially Allergic Patients
Intraepithelial cells				
Total cells, per mm*	39.3 ± 6.6	53.6 [†] ± 6.0	72 [‡] ± 8.6	24.6 ± 6.9
Mononuclear cells, per mm*	39.1 ± 6.5	48.3 [†] ± 5.2	58 ± 4.6	21.4 ± 5.9
Goblet cells, per mm*	20.4 ± 5.83	27.4 ± 6.8	36.1 ± 13.7	24.9 ± 9.5
Subepithelial collagen, per mm	0.01	0.01	0.01	0.01
Mucoserous glands				
(% of 1 mm²)*	31.3 ± 1.2	23 ± 0.04	25.1 ± 2.7	28 ± 2

* Mean ± SEM.

[†] Significantly different from perennial.

[‡] Significantly different from normal.

16-fold increase in tissue eosinophils, a 1.5-fold increase in mononuclear cells, and a 1.8-fold increase in total cell count from baseline. These subjects showed significantly more tissue eosinophils, neutrophils, and total cells than the unchallenged perennially allergic group. Despite the dramatic increase in eosinophils, the mononuclear cell remained the dominant cell in tissue by a factor of about tenfold. Histologic studies done by Vaheri support our findings that the predominant cellular infiltrate in the nasal mucosal stroma is mononuclear, and the neutrophilic granulocytes are present only in small numbers (18).

Migration of inflammatory cells through tight epithelial junctions occurs via cytomembrane processes that interdigitate with the epithelium. The mechanism of cellular compartmentalization in the nasal mucosa, however, is not yet fully understood. Cellular differences between the secretion and mucosa may imply differing cellular roles in nasal physiology and disease. For example, several authors have observed migration of basophils into the nasal secretions and of mast cells into the epithelium after antigen exposure (19–24).

The differences in the response of symptomatic, seasonally allergic subjects challenged with antigen and perennially allergic rhinitis subjects bear comment. Placing antigen at a site and being able to biopsy that same site 24 h later provides a favorable opportunity to observe changes. In perennial disease, exposure to the antigen may be more diffuse or lower in dose, or may represent a different stage of inflammation (i.e., hours versus months of exposure). Additional studies are needed to clarify the differences between the responses of perennially allergic and of seasonally allergic subjects to antigen challenge.

We found significant increases in the number of tissue eosinophils, mononuclear cells, and total cells only at the site of challenge. These data are consistent with the results of Connell and others, and do not support a centrally mediated cellular infiltration induced by neural reflex (25–27).

The eosinophil is considered to be an important cell in allergic rhinitis and asthma. Secretory products such as major basic protein and eosinophil cationic protein are both powerful toxins that can cause destruction and desquamation of the epithelium. The epithelium, which acts as a physicochemical barrier, is disrupted in asthma, and the disruption correlates with the degree of eosinophil infiltration (28–31). We found that the epithelium of seasonally allergic subjects was not damaged despite the influx of eosinophils 24 h after antigen challenge. In fact, the epithelium remained thicker and exhibited more stratified squamous “metaplastic” change. Varney and colleagues made the same observation when they showed that seasonally allergic subjects had no differences in epithelial thickness in biopsies performed 24 h after control or antigen challenges (14). Interestingly, their epithelial thickness measurement was similar to one reported in this study, despite biopsy of a different site. The epithelium was thicker than normal, and there was no difference in epithelial thickness between the ipsilateral and contralateral side of the nose following unilateral nasal antigen challenge. Furthermore, the number of eosinophils in the mucosa did not correlate with epithelial thickness, suggesting that this cell type was not intimately involved in the process affecting epithelial thickness. More subtle forms of injury may have been observed through other techniques or after more prolonged exposure. Locewicz, who obtained biopsy specimens before and after antigen challenge, also concluded that nasal epithelial cell damage and detachment are not a feature of allergic rhinitis (6).

We chose to biopsy the anterior tip of the inferior turbinate, rather than an area deeper into the nasal cavity. While the latter consistently yields ciliated epithelium, we were more interested

in a dynamic area of epithelium affected by antigen. The dynamic nature of the anterior tip of the inferior turbinate is highlighted by several observations. First, the bulk of inspired air passes over the anterior tip, causing pollen grains to impact on the tip (32). Second, biopsies of nasal mucosa in patients before and 60 d after total laryngectomy, a procedure eliminating nasal airflow, show a shift from squamous to ciliated cells (33). Third, the continuous use of intranasal steroids in patients with perennial rhinitis tends to shift the epithelium of the anterior nasal cavity from squamous to ciliated epithelium (34). Thus, the anterior tip of the inferior turbinate represents a dynamic area affected by allergic inflammation, and is an excellent site at which to study the allergic reaction.

Although acute challenges did not change the epithelium, epithelial attrition was evident in the mucosa of the perennially allergic group of subjects. Seven of 18 subjects displayed prominent epithelial thinning. Likewise, Spector, on examining nasal biopsies of perennially rhinitic individuals, noted that their epithelium varied between pseudostratified ciliated columnar and stratified squamous epithelium (33). Epithelial changes may therefore require more prolonged inflammatory exposure.

The basement membrane, identified by PASD staining, is visualized with the light microscope as a fine pink line coursing directly beneath the mucosal epithelium. It is composed mainly of type IV collagen, a glycoprotein called laminin, and a heparin sulfate-rich proteoglycan called BM-1. Collagen type IV is distributed on epithelial and endothelial basal laminae and basement membranes. This type of collagen is a thin, amorphous, weakly birefringent membrane and does not form fibrils or fibers. It functions mainly for support and filtration. Numerous studies of bronchial asthma have reported extensive deposition of collagen beneath the epithelial basement membrane of asthmatic patients (35–38). Connell, after repetitive nasal antigen challenge, observed destruction of the basement membrane, with a marked decrease in ground substance and collagen and with a marked infiltrate of reticular cells as compared with the findings in the unchallenged nostril (25). We did not find any significant differences in subepithelial collagen thickening among the different groups of subjects before or after antigen provocation, nor was there disruption of the PASD-positive basement membrane. There was no evidence of destruction or thickening of the subepithelial collagen even in the perennially allergic subjects.

The mucus-producing elements in the respiratory tract are the goblet cells and mucoserous glands. Karlsson and Pipkorn found no differences in the density of goblet cells before and during the allergy season (39). Murray found no relationship between the density of goblet cells and eosinophilia and signs and symptoms of allergic rhinitis (40). Tos and Mogensen observed that goblet cells are four times more numerous in pseudostratified epithelium than in transitional epithelium, and that their distribution is extremely irregular (41, 42). They also reported that subjects with nasal allergy had a significantly greater density of mucoserous glands than did normal subjects (43). We found no significant differences in the density of goblet cells and mucoserous glands among the groups.

In summary, the nasal secretion and nasal mucosa appear to represent two distinct compartments with differing leukocyte populations. Following antigen challenge, the predominant leukocyte in the nasal lavage is the neutrophil, while the predominant leukocyte in the mucosa is the mononuclear cell. Significant increases in tissue eosinophils, mononuclear cells, and total cell count occur only at the site of challenge. Seasonally allergic subjects had significantly thicker epithelium and a greater number of intra-

epithelial mononuclear cells and total cells than did perennially rhinitic and normal subjects. We did not find epithelial destruction, thickening of the epithelial basement membrane, or subepithelial collagen on antigen exposure, as described in bronchial asthma. Furthermore, our data do not support the role of the eosinophil in epithelial destruction.

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