

Clinical aspects of allergic disease

Nasal polyposis: Immunohistochemistry and bioelectrical findings (a hypothesis for the development of nasal polyps)

Joel M. Bernstein, MD, PhD,^a Janet Gorfien, MS,^b Bernice Noble, PhD,^b and James R. Yankaskas, MD^c Buffalo, N.Y., and Chapel Hill, N.C.

Nasal polyps and turbinates were obtained from individuals undergoing surgery for symptomatic nasal obstruction caused by nonatopic rhinosinusitis or allergic rhinosinusitis. One part of the tissue from each patient was fixed in neutral buffered formalin and prepared for study by histochemical and immunohistochemical methods. Monoclonal antibodies were used to identify macrophages, lymphocytes, and plasma cells. In most cases (12 of 16, 75%) the remainder of the polyp and turbinate samples was treated with protease to achieve disaggregation of the epithelial cells. Those cells were cultured on permeable collagen matrix supports. Transepithelial potential difference and resistance were measured daily. At the time of maximal transepithelial potential difference, the epithelial cells were mounted in modified Ussing chambers and exposed to a sodium-positive channel blocker (amiloride hydrochloride) and to selected chloride-negative channel agonists (isoproterenol bitartrate and adenosine triphosphate). Middle turbinates and polyps were found to have more macrophages, lymphocytes, plasma cells, HLA-DR-positive cells, and eosinophils than the inferior turbinates. Epithelial cells obtained from polyps exhibited higher transepithelial potential differences and equivalent short-circuit currents than turbinate cell cultures. The responses to amiloride, isoproterenol, and adenosine triphosphate were also greater for polyp than for turbinate cultures. A theory for the pathogenesis of nasal polyps is proposed. Local release of inflammatory mediators could cause sodium absorption and chloride permeability to be higher in polyps than in turbinate epithelia. Increased sodium absorption is consistent with the hypothesis that epithelial fluid absorption contributes to the development of nasal polyps and is a result of the increased recruitment of inflammatory cells, which are present in nasal polyps. (J Allergy Clin Immunol 1997;99:165-75.)

Key words: Nasal polyps, inferior turbinate, immunoglobulin, sodium channel, chloride channel, macrophage, plasma cells, colony-stimulating factors, glucocorticoids, secretory component

The exact pathogenesis of nasal polyps is unknown. Several theories have been formulated over the last 20 years, which attribute nasal polyposis to a variety of causes including allergy,¹ genetic predisposition,²⁻⁵ autonomic dysfunction of the blood vessels of the nasal mucosa,⁶ and inflammation.⁷

In the last decade, cytokine production by inflammatory and structural cells within nasal polyps has been demonstrated.⁸⁻¹⁵ These cytokines could recruit, activate, and prolong the survival of mononuclear and polymorphonuclear inflammatory cells within the pol-

Abbreviations used

Ieq:	Equivalent short-circuit current
R _t :	Transepithelial resistance
V _t :	Transepithelial potential difference

yps. Similar mechanisms have been suggested to operate in asthma and allergic rhinitis.

Previous reports from our laboratory have demonstrated that defects in sodium ion flux may cause water retention in nasal polyps both from patients with cystic fibrosis and patients who do not have cystic fibrosis.^{16, 17} We have proposed that the abnormal concentrations of inflammatory mediators present in nasal polyps might influence the function of both sodium and chloride channels at the luminal surfaces of the nasal epithelium and nasal polyps.¹⁸⁻²⁰ Modulation of ion flux by inflammatory mediators could lead to water retention and edema of the nasal mucosa and contribute to the development of nasal polyps. In our previous reports, however, we did not correlate the presence of inflamma-

From ^aDepartments of Otolaryngology and Pediatrics, State University of New York at Buffalo, Department of Communicative Disorders and Sciences, State University of New York at Buffalo, and the Division of Infectious Diseases, Children's Hospital of Buffalo; ^bDepartment of Microbiology, State University of New York at Buffalo; and ^cDivision of Pulmonary Diseases, Department of Medicine, University of North Carolina at Chapel Hill.

Received for publication Dec. 5, 1994; revised May 16, 1996; accepted for publication June 6, 1996.

Reprint requests: Joel M. Bernstein, MD, PhD, 15 South Forest Rd., Williamsville, NY 14221.

Copyright © 1997 by Mosby-Year Book, Inc.
0091-6749/97 \$5.00 + 0 1/1/75645

TABLE I. Mouse monoclonal antibodies used in this study

Antigen	Manufacturer	Specificity	Dilution
IgA ₁	Sigma	IgA ₁ immunocytes	1:1600
IgG ₁	Sigma	IgG ₁ immunocytes	1:100
IgG ₂	Sigma	IgG ₂ immunocytes	1:100
IgG ₃	Sigma	IgG ₃ immunocytes	1:800
IgG ₄	Sigma	IgG ₄ immunocytes	1:200
IgA ₂	Zymed	IgA ₂ immunocytes	1:200
IgD	Dako Corp.	IgD immunocytes	1:50
Secretory component	Dako Corp.	Free & bound secretory component	1:75
MAC 387	Dako Corp.	Macrophage	1:800
CD 68	Dako Corp.	Macrophage	1:800
IgM	Dako Corp.	IgM immunocytes	1:800
HLA-DR	Dako Corp.	Antigen-presenting cells	1:400
L 26	Dako Corp.	B cells	1:400

TABLE II. Criteria for scoring of immunohistochemical observations

Region	Score	Description
Epithelium	0	No stained cells
	+	Some cells stained
	++	All cells stained
	+++	All cells very stained
Basement membrane	0	No deposits
	+	Occasional deposits
	++	Moderate deposits
	+++	Heavy deposits
Blood vessels	0	No transudate
	+	Some accumulation
	++	Moderate accumulation
	+++	Heavy accumulation
Serous acinar cells	0	No stained glands
	+	Some glands stained
	++	All glands stained
	+++	All glands very strongly stained

tory cells with the changes in the bioelectrical parameters of the nasal polyps or the corresponding inferior turbinate mucosa. In this study we have asked whether the inflammatory cell population in polyps is different from that of the inferior turbinates and whether the cells that are recruited into the nasal polyp might affect local pathophysiology.

The purpose of this report is to present new information on the distribution of lymphocytes and macrophages in nasal polyps and middle and inferior turbinates and to correlate bioelectrical events in vitro with inflammatory phenomena. On the basis of this new information and a review of the literature, a hypothesis for the pathogenesis of nasal polyposis is proposed.

METHODS

Histologic and immunocytochemical studies

Tissue samples were obtained from 16 patients (8 female and 8 male), aged 16 to 74 years, (mean age, 44 years; SEM, 14), who underwent surgery for treatment of nasal polyps. Four patients had IgE-mediated hypersensitivity diagnosed on the basis of at least three positive skin test results for inhalant allergy; 12 were considered nonallergic. None had been using locally applied corticosteroids for at least 6 weeks before surgery.

Biopsy specimens were taken from the medial aspect of the middle turbinate and from the inferior turbinate about 1.5 cm from its anterior tip. Polyps were taken from the lateral wall of the nose, lateral to the middle turbinate.

The specimens were fixed in 10% neutral buffered formalin for 24 to 48 hours, washed, processed through a series of graded alcohols, cleared in toluene, and embedded in paraffin. Sections were cut at 4 μ m and dried in a 37° C oven overnight. Routine staining with hematoxylin and eosin, as well as vital new red stain,²¹ was done on all tissues for visualization of eosinophils.

The mouse monoclonal antibodies used for immunohistochemical staining in this study are listed in Table I. Tissue sections were deparaffinized with xylene and rehydrated through a series of graded alcohols to distilled water. When necessary, the sections were treated with 0.1% trypsin (Sigma Chemical Co.) in a 0.1% calcium chloride solution for 30 minutes at 37° C. Endogenous peroxidase activity was blocked by treatment with a 0.05% 4-chloro-1-naphthol solution in 0.05 mol/L Tris-HCl buffer (pH 7.65) and 0.5% ethanol and 0.05% H₂O₂ for 15 minutes. Antigen retrieval was performed with a 10 mmol/L citrate buffer (pH 6.0) to determine whether antibody staining could be enhanced. It was concluded that antigen retrieval did not significantly change the staining patterns, and for that reason, it was not routinely used except as noted in the Results section.²² An Avidin-Biotin-Complex staining kit (Vector Laboratories, Burlingame, Calif.) was used for the remainder of the staining process. 3,3'-Diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) was used for the final color reaction, after which the sections were counterstained with Mayer's hematoxylin (Sigma Chemical Co.) for 1 to 2 minutes. Human tonsil tissue served as a positive control. For negative controls, the primary antibody was omitted.

Positively stained cells were counted separately in each of six

TABLE III. Immunoglobulin subclass localization

Antibody	Serous acinar cells	Respiratory epithelial cells	Basement membrane deposits	Blood vessel walls
IgG ₁				
Inferior Turbinates	++	+++	+	+++
Middle Turbinates	++	+++	+	+++
Polyps	+	+++	+	++
IgG ₂				
Inferior Turbinates	++	+++	+	+++
Middle Turbinates	++	+++	+	+++
Polyps	++	+++	+	+++
IgG ₃				
Inferior Turbinates	0	0	+	0
Middle Turbinates	0	0	+	+
Polyps	0	0	+	+
IgG ₄				
Inferior Turbinates	0	++ (40%)	0	0
Middle Turbinates	0	++ (40%)	0	0
Polyps	0	++ (60%)	+	0
IgA ₁				
Inferior Turbinates	+	+	+	+
Middle Turbinates	+	+	+	+
Polyps	+	+++	+	+
IgA ₂				
Inferior Turbinates	+	+++	+	0
Middle Turbinates	+	+++	+	0
Polyps	+	+++	+	0

Data in this table are mode scores. With most of the anti-immunoglobulin antibodies used in this study, staining patterns were similar in all biopsy specimens. The data in this table represent the mode score (see Methods) for the extent of staining in each category. When presented without a percentage in parentheses, that mode score was based on 100% of the biopsy specimens. In those instances in which positive staining for a particular immunoglobulin subclass was noted in some but not all biopsy specimens, the percentage of positive biopsy specimens has been noted in parentheses. The mode score reported in this table for those categories was based only on positive samples.

distinct histologic compartments: the respiratory epithelium, the basement membrane, the lamina propria, the superficial and deep glandular epithelium (serous and mucous), the periglandular connective tissue, and the blood vessels. For each compartment a minimum of 10 high-power fields ($\times 400$) were counted. A semiquantitative scoring system used to record the observations is listed in Table II. For the IgG and IgA subclasses in the three major tissue areas, the mode, or most frequent score in each category has been reported here to represent the best staining observed (Table III). When deep glands were present in the biopsy specimen of the middle or inferior turbinates, both the superficial and deep glands were evaluated separately. Because no significant differences were noted, observations of all serous acinar cells have been reported together.

Measurement of bioelectrical properties of cultured nasal polyp and turbinate epithelial cells

Bioelectric properties of cultured human nasal polyp and turbinate epithelial cells were measured in specimens from 12 patients for whom immunohistologic studies had also been performed.

Epithelial cells were isolated from polyp and inferior turbinate samples by protease dispersion and cultured on permeable collagen-matrix support dishes, as previously described.²³ After the cells appeared confluent by phase-contrast microscopy,

transepithelial potential difference (V_t) was measured daily by using a high input impedance volt meter connecting the mucosal and submucosal solutions through sterile calomel half cells. Transepithelial resistance (R_t) was calculated from the change in voltage induced by 500-millisecond constant current pulses (generally $99 \mu\text{A}/\text{cm}^2$). Tissue was ohmic over the range of -40 to $+40$ mV. The equivalent short-circuit current (I_{eq}) was calculated from V_t and Ohm's Law. The epithelial cell cultures reached maximal V_t 2 to 14 days after seeding (6.3 ± 0.4 days, $n = 46$). Within 2 days of reaching maximal V_t , the cells were transferred to Ussing chambers for evaluation of mediator responses as described by Willumsen et al.²⁴ The mucosal and submucosal surfaces of the cultures were bathed in Krebs-Ringer bicarbonate buffer solutions, which had been warmed to 37°C and gassed with 95% O_2 and 5% CO_2 . The V_t was measured by a VCC 600 electrometer (Physiologic Instrument, San Diego, Calif.) connected to the half chambers by calomel electrodes and 3 mol/L KCl agar bridges. The V_t was clamped to 0 mV by passing current through Ag-AgCl electrodes and Krebs-Ringer bicarbonate buffer agar bridges. The short-circuit current was recorded, and the circuit was interrupted intermittently to record V_t . Ten to twenty minutes after mounting, $60 \mu\text{l}$ aliquots of $100\times$ stocks of drugs were sequentially added to the 6 ml mucosal bath to achieve the following final concentrations: amiloride 10^{-4} mol/L, isoproterenol 10^{-5} mol/L, and adenosine triphosphate 10^{-4} mol/L.

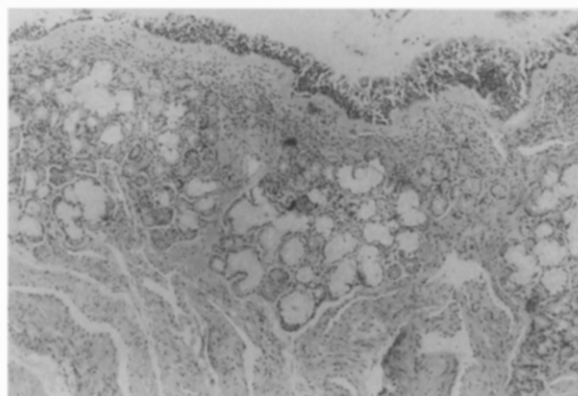


FIG. 1. Section of inferior turbinate stained with hematoxylin and eosin. Typical structure of the inferior turbinate is shown with pseudostratified columnar ciliated epithelium and typical seromucous glands. Note the large arterioles at the bottom of the photograph. (Hematoxylin and eosin, $\times 100$.)

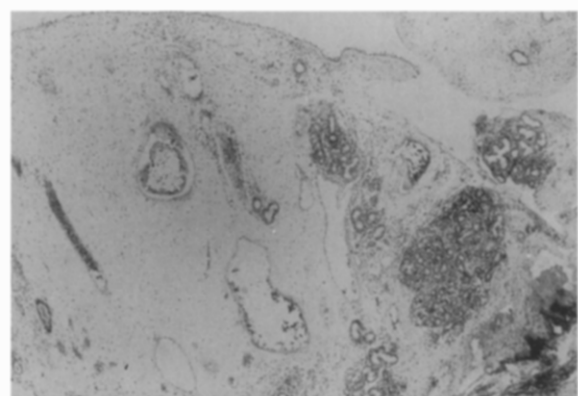


FIG. 2. Photomicrograph of polyp arising from middle turbinate mucosa. On the right are typical seromucous glands found in the middle turbinate. Adjacent to these glands, on the left, are large cystically dilated abnormal glands of a nasal polyp with tremendously edematous stroma.

RESULTS

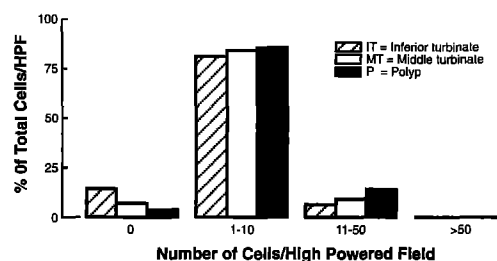
Conventional histologic examination

Inferior turbinates. As determined by conventional histologic examination, the inferior turbinates contained either stratified squamous epithelium or pseudostratified columnar ciliated epithelium, under which a thickened basement membrane was noted in 70% of the samples. Multiple seromucous glands, present in both superficial and deep layers, were separated by large venous sinusoids (Fig. 1).

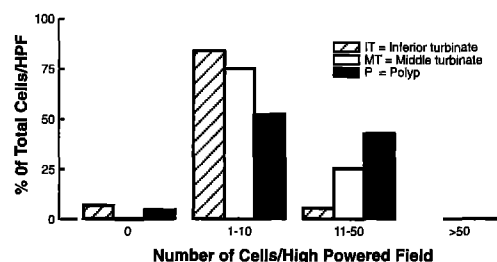
Middle turbinates. The histologic appearance of the middle turbinates was similar to that of the inferior turbinates, but with more inflammatory cells. The middle turbinate contained pseudostratified columnar ciliated epithelium, a classical respiratory epithelium.

Nasal polyps. The histologic appearance of nasal polyps varied, from extremely edematous tissue with very few glands, to tissue that had scattered glands. The

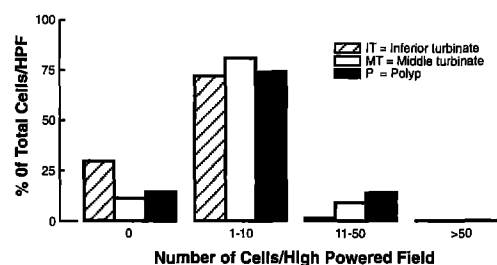
A.



B.



C.



D.

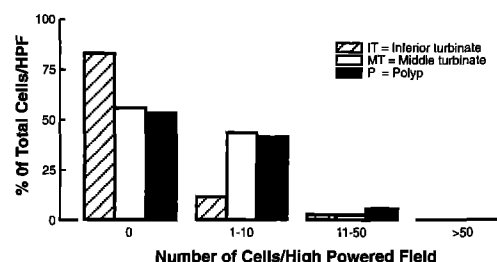


FIG. 3. Periglandular distribution of cells in different locations of nasal mucosa involving IgG subclasses (A, IgG₁; B, IgG₂; C, IgG₃; D, IgG₄). The graphs represent a semiquantitative distribution of immunocytes in the inferior turbinate, middle turbinate, and polyp and demonstrate the number of cells per high-power field related to the percentage of total high-power fields. In general, there are more IgG₁ cells and IgG₂-positive cells in polyps than in inferior turbinates. HPF, High-power fields.

unique features of the polyps were: (1) an irregularity of the structure of the cystically dilated glands, (2) a virtual absence of the true seromucous glands as found in the inferior and middle turbinates, and most importantly,

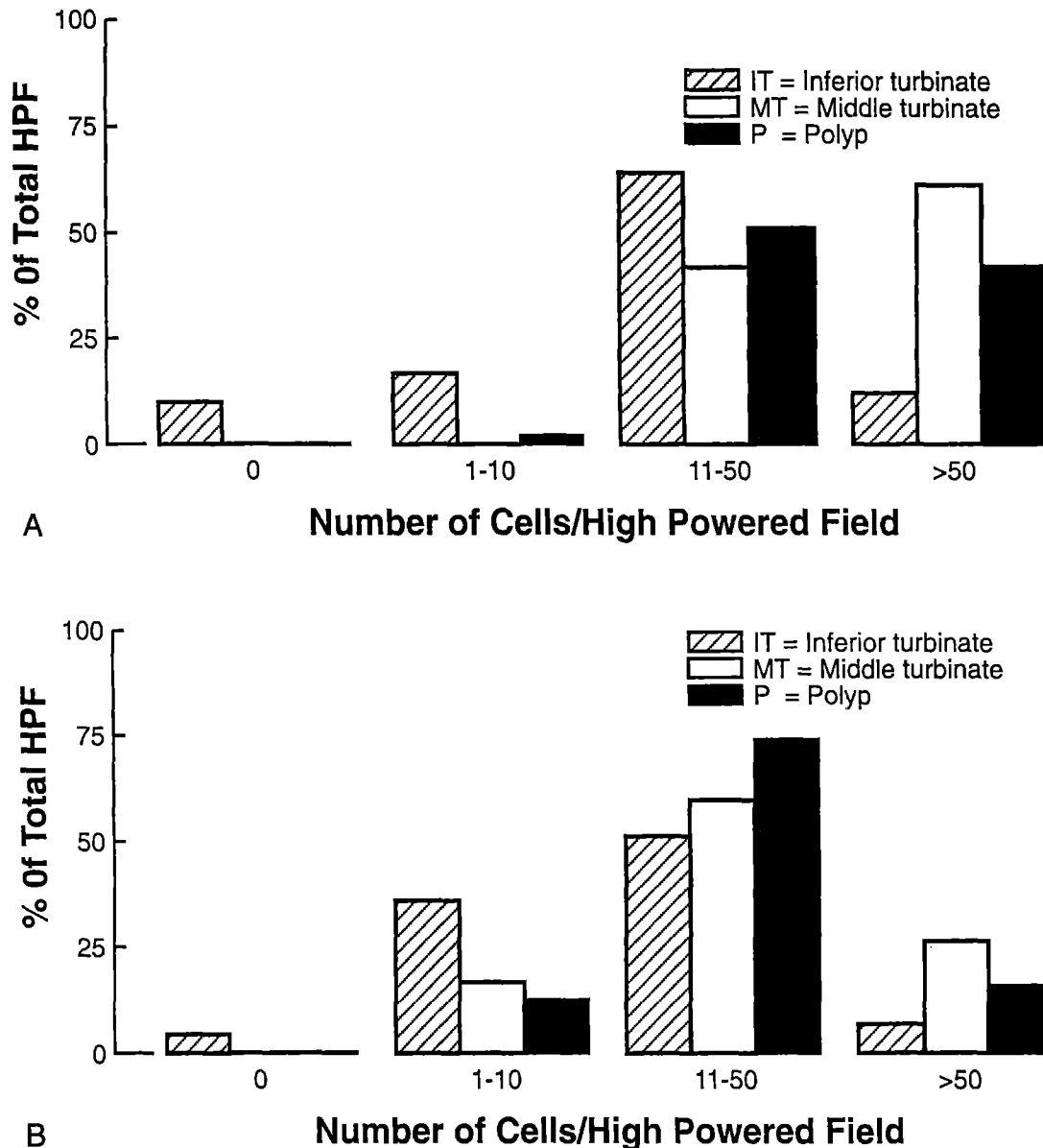


FIG. 4. Periglacial distribution of IgA₁ (A) and IgA₂ (B) subtypes in the inferior turbinate, middle turbinate, and nasal polyps. Many more IgA-positive plasma cells are present in middle turbinates and polyps compared with inferior turbinates. HPF, High-power fields.

(3) a large accumulation of inflammatory cells, particularly eosinophils (Fig. 2).

Immunohistologic examination of inferior turbinates

IgG. IgG₁ and IgG₂ were present in respiratory epithelial cells, acinar cells of the seromucous glands, and blood vessel walls (Table III). IgG₁- and IgG₂-positive plasma cells were observed in low frequency in periglacial regions (Fig. 3, A and B). Accumulation of IgG₃ and IgG₄ was, for the most part, minimal or absent except that: (1) IgG₄ was noted in the respiratory epithelium of many samples, and (2) IgG₃-positive

plasma cells were as frequent as IgG₁ and IgG₂ in the periglacial region.

IgA. IgA₁ and IgA₂ were readily detected in respiratory epithelial cells of the inferior turbinates; staining for IgA₂ was more intense and more frequent than that for IgA₁ (Table III). IgA₁-positive plasma cells were observed much more frequently than IgG plasma cells around the seromucous glands (Fig. 4, A and B). The total number of cells positive for IgA₁ and IgA₂, evaluated with subset-specific antibodies, was equal to the number stained with a pan IgA antibody. IgA₁-staining immunocytes, however, were much more common in the periglacial areas (Fig. 5).

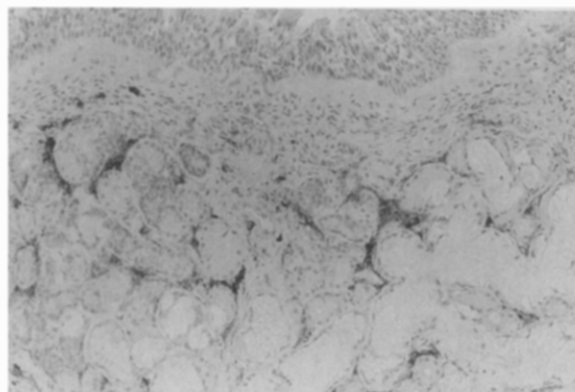


FIG. 5. Distribution of IgA₁ in the inferior turbinates. There are significant numbers of IgA₁-positive plasma cells surrounding the serous glands in the lamina propria of the inferior turbinate. (Immunoperoxidase, $\times 200$.)

IgM and IgD. Blood vessel walls of the inferior turbinates were frequently positive for IgM. IgM- and IgD-positive plasma cells were sparsely scattered throughout the lamina propria. A low frequency (less than 10 per high-power field) of IgD plasma cells was also observed in the periglandular regions.

Secretory component. Cells of the respiratory epithelium and acini of the seromucous glands were intensely stained with antibody to secretory component (Fig. 6).

B cells. L26-positive B cells, sparsely scattered (less than 10 per high-power field) in the lamina propria, were absent in periglandular regions.

Macrophages. The lamina propria of the inferior turbinates contained low numbers of macrophages (less than 10 per high-power field) (Fig. 7, A).

HLA-DR (Class II). Cells of the respiratory epithelium and serous acini did not express Class II antigens. HLA-DR-positive mononuclear cells found in connective tissue surrounding the glands were few in number (Fig. 7, B). Even with antigen retrieval methods, cells of the respiratory epithelium did not stain with antibodies to HLA-DR. The number of positive mononuclear cells in periglandular areas and in the lamina propria was not significantly increased by antigen retrieval.

Eosinophils. Rare in the inferior turbinates, eosinophils were limited to the lamina propria.

Middle turbinates

IgG. The IgG distribution in middle turbinates was very similar to that in the inferior turbinates, although IgG₂ and IgG₄ plasma cells were slightly more frequent (Fig. 3, B and D).

IgA. Staining for IgA in the middle turbinates was also similar to that for the inferior turbinates, except in the periglandular regions (Fig. 4, A and B). Both IgA₁- and IgA₂-positive plasma cells were detected with greater frequency in the middle turbinates than in the inferior turbinates, with IgA₁-positive immunocytes predominating.

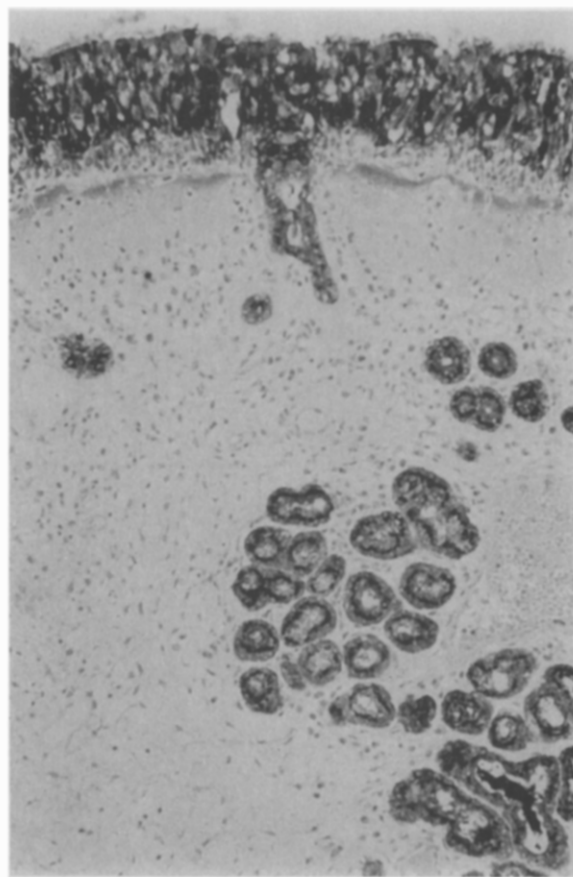


FIG. 6. Distribution of secretory component in inferior turbinate showing dense accumulation of stain in epithelial cells of the surface and acinar cells of the superficial glands. (Immunoperoxidase, $\times 200$.)

IgM and IgD. IgM-positive cells were increased in number in interepithelial sites of middle turbinates compared with inferior turbinates. Otherwise, patterns of IgM and IgD positivity were similar in middle and inferior turbinates.

Secretory component. The distribution in middle turbinates was not different from that in inferior turbinates.

B cells. The distribution of B cells in the middle turbinates was similar to that of the inferior turbinates.

Macrophages. Macrophages were more frequent in the middle turbinate tissue samples than in inferior turbinates, especially in connective tissue, interepithelial sites, and periglandular areas (Fig. 7, A).

HLA-DR. The distribution of HLA-DR-positive cells in the middle turbinates was similar to that in the inferior turbinates, but HLA-DR-positive cells were more numerous in the lamina propria below the respiratory epithelium and in the periglandular sites (Fig. 7, B).

Eosinophils. Eosinophils were more frequent than in the inferior turbinates, although no high-power field had more than 50 cells (Fig. 8).

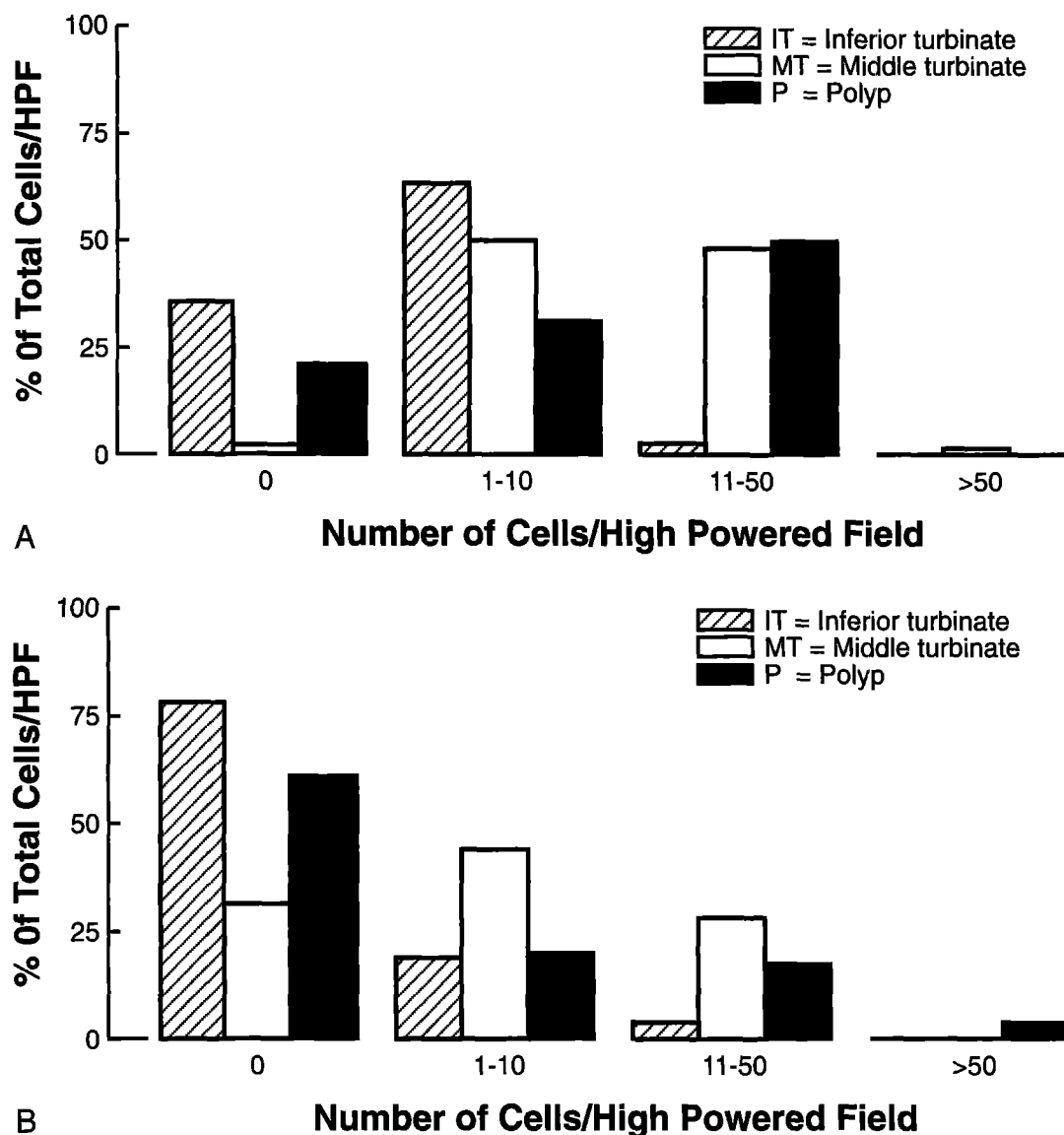


FIG. 7. A, Periglandular distribution of MAC 387 cells in the inferior turbinate, middle turbinate, and nasal polyps. There are greater numbers of macrophages in the inferior turbinate and the polyps than in the middle turbinate. **B,** Periglandular distribution of HLA-DR in the inferior turbinate, middle turbinate, and nasal polyp. Only in the middle turbinate and nasal polyp are significant numbers of HLA-DR cells distributed. *HPF*, High-power fields.

Nasal polyps

IgG. The distribution pattern of IgG in polyps was similar to that in the turbinates, although plasma cell numbers were higher in periglandular regions (Fig. 3, *A-D*).

IgA. Nasal polyps contained more IgA plasma cells than either middle or inferior turbinates, with IgA₁ predominating (Fig. 4, *A* and *B*).

IgM and IgD. The distribution of IgM and IgD in polyps was essentially indistinguishable from that in middle turbinates; a slight increase of IgM plasma cells was detected in the lamina propria and periglandular sites.

Secretory component. Secretory component was detected in acinar cells and in the respiratory epithelium of nasal polyps. A pattern similar to that observed in middle turbinates occurred except that the epithelium was much less intensely stained in polyps (Fig. 9).

B cells. The distribution and frequency of B cells in polyps was similar to that in the middle turbinate.

Macrophages. Macrophages were more frequent in interepithelial sites in the polyps than in the turbinates (Fig. 10). Especially striking was margination within the blood vessels of MAC387-positive monocytes (Fig. 11).

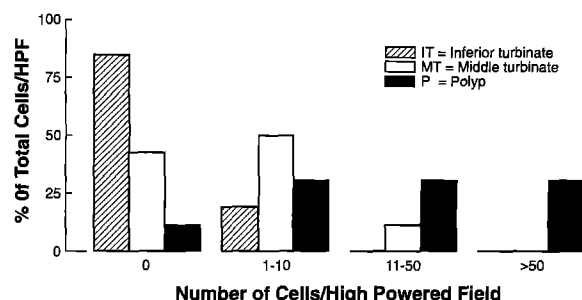


FIG. 8. Connective tissue distribution of eosinophils in nasal mucosa. A marked increase in eosinophils is present in nasal polyps compared with both the middle and inferior turbinates. *HPF*, High-power fields.

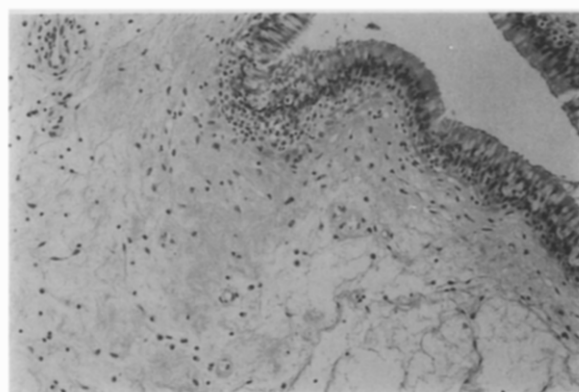


FIG. 9. Distribution of secretory component in a nasal polyp. There is a decrease of secretory component in the epithelial cells of this polyp. (Immunoperoxidase, $\times 200$.)

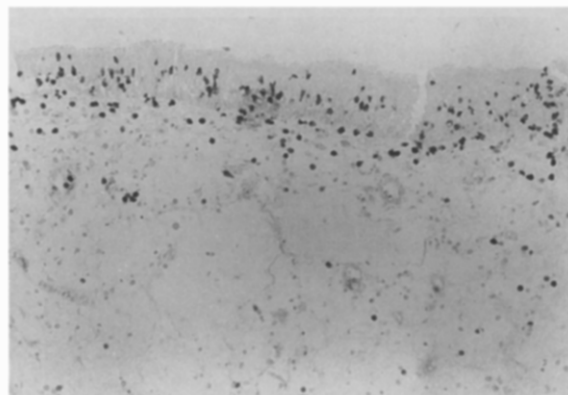


FIG. 10. MAC 387 staining for macrophages in the nasal polyp showing significant staining of interepithelial cells, as well as in the lamina propria. (Immunoperoxidase, $\times 200$.)

HLA-DR. The distribution of HLA-DR-positive cells in the polyps was similar to that in the middle turbinates. The periglandular distribution of HLA-DR-positive cells is summarized in Fig. 7, *B*.

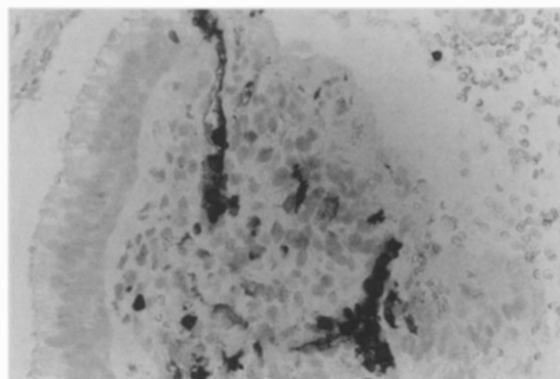


FIG. 11. MAC 387 staining in a nasal polyp showing significant margination of monocytes in blood vessels. (Immunoperoxidase, $\times 400$.)

TABLE IV. Maximal bioelectric properties of cultured human nasal polyp and turbinate epithelial cells

	<i>n</i>	V_t (mV)	R_t (Ω/cm^2)	I_{eq} ($\mu\text{A}/\text{cm}^2$)
Polyp (12 patients)	37	-11.2 ± 1.5	243 ± 15	43.6 ± 4.2
Turbinate (7 patients)	9	-5.3 ± 1.4	187 ± 24	28.5 ± 5.7

Eosinophils. Large numbers of eosinophils were found in polyps, and extensive degranulation was a common observation (Figs. 8 and 12).

Bioelectric properties of cultured polyp and turbinate epithelial cells

Most polyp specimens produced epithelial cell cultures with measurable and significant bioelectric properties (i.e., $R_t > 90 \Omega/\text{cm}^2$). The cell yield from turbinate specimens was about 30% lower, and the number of individual cultures that developed a significant R_t was also lower. The maximal V_t , R_t , and I_{eq} values of these cultures (Table IV) are comparable to those previously reported.¹⁷ The most striking observation was the increased V_t and I_{eq} in polyp cells, as compared with turbinate cells.

To evaluate the regulatory pathways for ion transport, specimens were evaluated in Ussing chambers in basal conditions and during exposure to selected chemicals. The overall results are summarized in Table V. The R_t of three turbinate cultures was lower than that of the polyp samples and the overall turbinate sample (Table IV), probably reflecting sample variation. The short-circuit current was significantly decreased by amiloride and was increased by both isoproterenol and adenosine triphosphate. Turbinate cultures had similar but smaller responses (Table V).

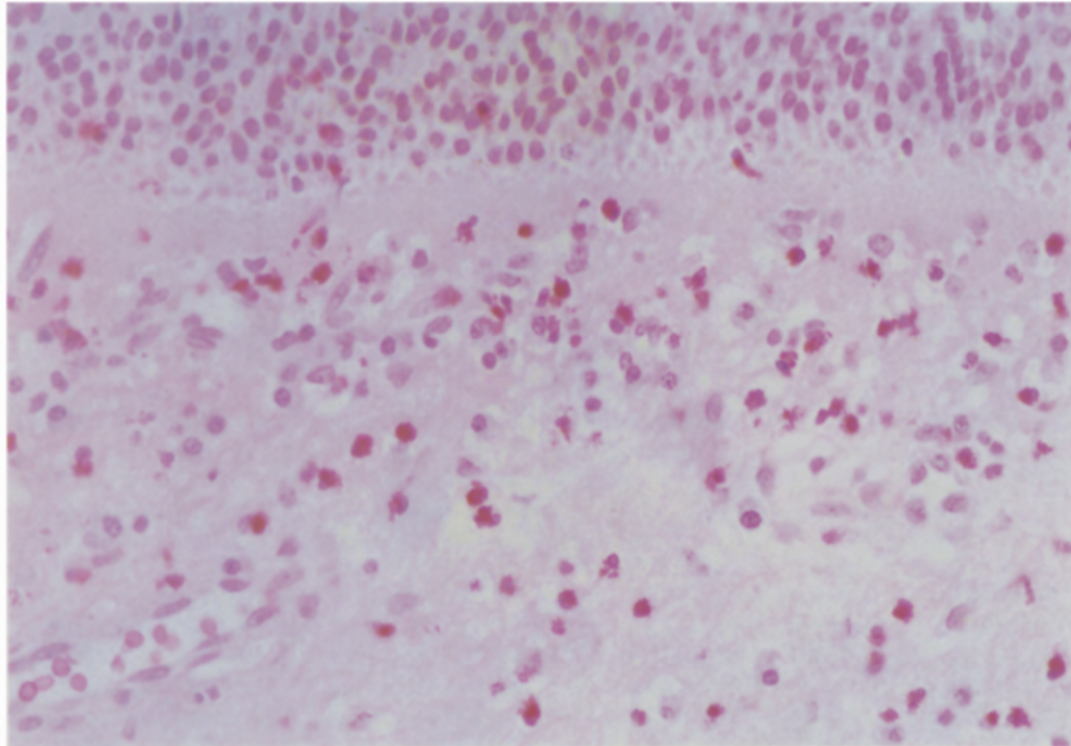


FIG. 12. Distribution of eosinophils in the lamina propria of the nasal polyp indicated with vital new red stain ($\times 400$). There are many degranulated eosinophils in the lamina propria.

TABLE V. Bioelectric properties of cultured human nasal polyp and turbinate epithelial cells

	Polyps ($n = 21$)			Turbinates ($n = 3$)		
	V_t (mV)	R_t (Ω/cm^2)	I_{eq} ($\mu\text{A}/\text{cm}^2$)	V_t (mV)	R_t (Ω/cm^2)	I_{eq} ($\mu\text{A}/\text{cm}^2$)
Basal	-7.8 ± 1.2	173 ± 16	44.1 ± 6.2	-2.4 ± 1.0	103 ± 17	20.8 ± 6.6
Amiloride (10^{-4} mol/L)	$-4.0 \pm 0.5^*$	$208 \pm 20^*$	$20.6 \pm 2.0^*$	-1.1 ± 0.3	104 ± 15	10.4 ± 1.5
Isoproterenol (10^{-5} mol/L)	$-4.3 \pm 0.5^*$	194 ± 15	$22.8 \pm 2.2^*$	-1.3 ± 0.4	100 ± 16	12.7 ± 2.9
ATP (10^{-4} mol/L)	$-6.7 \pm 1.1^*$	$142 \pm 13^*$	$64.0 \pm 11.4^*$	-1.7 ± 1.8	77 ± 15	30.6 ± 11.1

Values are expressed as means $\pm$ SEM.

ATP, Adenosine triphosphate.

*Significant change from preceding value, $p < 0.01$, paired t test.

DISCUSSION

Numerous hypotheses have been formulated to explain the cause of nasal polyps. Although theories involving dysfunction of the autonomic nervous system of the nose⁶ and abnormalities of carbohydrate metabolism²⁵ and vasomotor imbalance²⁵ have been suggested, the two most plausible, widely discussed theories involve allergy¹ and inflammation.⁷ Mechanisms other than allergy, however, appear to be involved in the accumulation of inflammatory cells in nasal polyps because of the observed increase in the number of mucosal mast cells in the nasal turbinate epithelium of patients with polyps who do not have IgE-mediated hypersensitivity²⁶ and

because of the epidemiologic data, which strongly suggest that nasal polyposis occurs more commonly in nonatopic patients than in atopic patients.²⁷

This study confirms and extends the observations of Tos et al.,²⁸ who demonstrated that polyps differ from normal mucosa in lacking seromucous glands and in possessing cystically dilated degenerated glands containing inspissated mucus. Furthermore, the predominant inflammatory cell in the nasal polyp is the eosinophil. The data from this study demonstrate a progressive increase in the number of mononuclear cells, antigen-presenting cells, and plasma cells, ascending in the nose from the inferior turbinate to the middle turbinate. Our

observations are similar to those of Stoop et al.,^{29, 30} who also suggested that local immunologic and inflammatory processes may play a prominent role in the development of nasal polyps. In their studies eosinophil infiltration was a striking feature of many nasal polyps; eosinophils were significantly more numerous in nasal polyps than in the mucosa of middle and inferior turbinates. Stoop et al.^{29, 30} have also identified an abundance of HLA-DR-positive cells in the stroma and epithelial regions of nasal polyps and middle turbinates compared with inferior turbinates. Our observations, in agreement with those of Stoop et al.,^{29, 30} also indicate that HLA-DR cells are more prominent in the lamina propria of nasal polyps than in the inferior turbinate mucosa, although we could not confirm the presence of HLA-DR-positive cells in the epithelia of either the middle turbinates or nasal polyps.

This study also corroborates the elegant findings of Bjerke and Brandtzaeg³¹ and Brandtzaeg.³² We observed a slightly higher frequency of IgA₂-positive immunocytes in our patients than has been described by Bjerke and Brandtzaeg, although IgA₁-positive cells were by far more common, as has been shown. Intracellular IgA₂ may have been especially well preserved in the plasma cell cytoplasm of the formalin-fixed tissues that were used for our studies. In the tonsil tissue that served as controls, the distribution and frequency of IgA₁ and IgA₂-positive cells were identical to those of previous reports,³² confirming the validity of reagents and tissue-processing protocols used in the study. Secretory component was not observed exactly in the same intracellular distribution as IgA₂.

The molecular biologic mechanisms by which macrophages, eosinophils, and lymphocytes are increased in nasal polyps are not known. However, Denberg et al.^{9, 13} have emphasized the importance of microenvironmental influences on airway inflammation. The investigations described here support the idea that inflammation is an important factor in the development of nasal polyposis. The number of macrophages, plasma cells, eosinophils, and basophils in nasal polyps is elevated compared with inferior turbinates. Those inflammatory cells increase the potential for immunologic reactivity in polyps compared with turbinates. Cytokines and growth factors released from structural cells of the nasal epithelium might also recruit inflammatory cells into the nasal polyp, although the precise order and sequence of inflammatory cell interactions in the development of nasal polyposis is not yet understood.

The data from our study suggest that nasal polyp epithelial cells have a normal luminal chloride channel, because isoproterenol, a drug that increases cyclic adenosine monophosphate and activates protein kinase A, increased chloride permeability (Table V). However, amiloride caused a greater decrease in sodium absorption in nasal polyp cells than in cells from inferior turbinate mucosa. Amiloride is a specific blocker of the luminal sodium channel and decreases the basal voltage, as well as basal equivalent current. These findings indi-

cate that sodium absorption may be increased in nasal polyps. The mediators released by inflammatory cells in nasal polyps could influence sodium absorption. Increased sodium absorption could lead to water retention in the epithelium and the lamina propria of polyps. The success of glucocorticoid treatment of nasal polyps may result, at least in part, from the inhibition of local cytokine secretion in nasal polyps.³³

Despite the fact that surgery can relieve nasal obstruction, there is a very high recurrence rate of polypoid mucous membrane in patients, particularly in patients with the triad of aspirin intolerance, chronic sinusitis, and bronchial asthma.³⁴ Polyp recurrence may be under genetic control.⁵

On the basis of the information presented in this study, we propose a multivariate theory for the pathogenesis of nasal polyps. Turbulent flow of air in the lateral wall of the nose or viral-bacterial-host interactions could produce inflammatory changes in the lateral wall of the nose.³⁵ Ulceration and prolapse of the submucosa with re-epithelialization and new gland formation may occur (theory of Tos et al.²⁸). The structural cells of the nasal polyp (including epithelial cells, vascular endothelial cells, and fibroblasts) all have the ability to produce messenger RNA for granulocyte-monocyte colony-stimulating factor,¹¹ which could represent a major amplification pathway of the inflammatory response. This microenvironmental structural inflammatory response in the nasal polyp in turn can affect the bioelectrical integrity of the sodium and chloride channels at the luminal surface of the respiratory epithelial cell. The change in the sodium absorption, which has been demonstrated in this study, may result in an increased movement of water into the cell and into the interstitial fluid. The resultant edema can lead to enlargement of the nasal polyp. Finally, the rapid recurrence of nasal polyps, despite adequate surgery, may reflect some intrinsic phenotypic characteristic of nasal epithelial cells in the lateral wall of the nose, which is likely to be under genetic control. The complex interaction of inflammatory mediators on nasal luminal epithelial cells has an effect on sodium absorption and chloride permeability. These events may result in recurrent development of edematous mucosal disease of the lateral wall of the nose, namely recurrent nasal polyposis.

We would like to acknowledge the Histology Laboratory, Department of Pathology, State University of New York at Buffalo for their technical assistance.

REFERENCES

1. Holopainen E, Mäkinen J, Paavolainen M, Palva T, Saldo DR. Nasal polyposis: relationship to allergy and acetyl salicylic acid intolerance. *Acta Otolaryngol* 1979;87:330-4.
2. Cookson WOC, Sharp PA, Faux JA, Hopkan JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. *Lancet* 1989;1(8650):1292-4.
3. Moloney JR, Oliver TD. HLA antigens, nasal polyps and asthma. *Clin Otolaryngol* 1980;5:183-9.

4. Drake-Lee A. Nasal polyps in identical twins. *J Laryngol Otol* 1992;106:1084-5.
5. Burger J, Macek M, Stuhmann M, Reis A, Krawczak M, Schmidt-ke J. Genetic influences in the formation of nasal polyps. *Lancet* 1991;337:974.
6. Cauna N, Hinderer KH, Manzetti CW, Swanson EW. Fine structure of nasal polyps. *Ann Otol Rhinol Laryngol* 1972;81:41-58.
7. Small P, Frenkiel S, Black M. Multifactorial etiology of nasal polyps. *Ann Allergy* 1981;46:317-20.
8. Jordana M, Vancheri C, Ohtoshi T, Harnish D, Gauldie J, Dolovich J, Denburg J. Hemopoietic function of the microenvironment in chronic airway inflammation. *Agents Action* 1989;28:85-95.
9. Denburg J, Dolovich J, Ohtoshi T, Cox G, Gauldie J, Jordana M. The microenvironmental differentiation hypothesis of airway inflammation. *Am J Rhinol* 1990;4:29-32.
10. Gauldie J, Jordana M, Cox G, Ohtoshi T, Dolovich J, Denburg J. Fibroblasts and other structural cells in airway inflammation. *Am Rev Respir Dis* 1992;145:14-7.
11. Ohtoshi T, Tsuda T, Vancheri C, Abrams J, Gauldie J, Dolovich J, et al. Human upper airway epithelial cell-derived granulocyte-macrophage-colony stimulating factor induces histamine-containing cell differentiation of human progenitor cells. *Int Arch Allergy Appl Immunol* 1991;95:376-84.
12. Ohnishi M, Ruhno J, Bienenstock J, Dolovich J, Denburg J. Hematopoietic growth factor production by cultured cells of human nasal polyp epithelial scrapings: kinetics, cell source, and relationship to clinical status. *J Allergy Clin Immunol* 1989;83:1091-100.
13. Denburg J, Gauldie J, Dolovich J, Ohtoshi T, Cox G, Jordana M. Structural-cell derived cytokines and allergic inflammation. *Int Arch Allergy Appl Immunol* 1991;94:127-32.
14. Ruhno J, Howie K, Andersson B, Vanzielegheem M, Hitch D, Lapp P, et al. The increased number of epithelial mast cells in nasal polyps and adjacent turbinates is not allergy-dependent. *Allergy* 1990;45:370-4.
15. Wong D, Jordana G, Denburg J, Dolovich J. Blood eosinophilia and nasal polyps. *Am J Rhinol* 1992;6:195-8.
16. Bernstein JM, Cropp GA, Nathanson I, Yankaskas JR. Bioelectric properties of cultured nasal polyp and turbinate epithelial cells. *Am J Rhinol* 1990;4:45-8.
17. Bernstein JM, Yankaskas JR. Increased ion transport and cultured nasal polyp epithelial cells. *Arch Otolaryngol Head Neck Surg* 1994;120:993-6.
18. Al-Bazzaz F, Yadava VP, Westenfelder C. Modification of Na⁺ and Cl⁻ transport in canine tracheal mucosa by prostaglandins. *Am J Physiol* 1981;240:101-5.
19. Leikauf GD, Ueki IF, Nadel JA, Widdicombe JH. Bradykinin stimulates chloride secretion and prostaglandin E₂ release by canine tracheal epithelium. *Am J Physiol* 1985;248:48-55.
20. Marin MG, Davis B, Nadel JA. Effect of histamine on electrical and ion transport properties of tracheal epithelium. *J Appl Physiol* 1977;42:735-8.
21. Duffy JP, Smith PJ, Crocker J, Matthews HR. Combined staining method for the demonstration of tissue eosinophils and mast cells. *J Histotechnol* 1993;16:143-4.
22. Tacha D, Chen T. Technical Note: Modified antigen retrieval procedure: calibration technique for microwave ovens. *J Histotechnol* 1994;17(no. 4):365-6.
23. Yankaskas JR, Cotton CU, Knowles MR, Gatzky JT, Boucher RC. Culture of human nasal epithelial cells on collagen matrix supports. *Am Rev Respir Dis* 1985;132:1281-7.
24. Willumsen JN, Davis CW, Boucher RC. Intra-cellular Cl⁻ activity and cellular pathways in cultured human airway epithelium. *Am J Physiol* 1989;256:1033-44.
25. Slavin RG. Nasal polyps and sinusitis. In: Middleton E Jr, Reed CE, Ellis CE, Atkinson NF Jr, Yunginger JW. *Allergy: principles and practice*. 3rd ed. St. Louis: CV Mosby, 1988:1291-303.
26. Ruhno J, Howie K, Andersson B, Vanzielegheem M, Hitch D, Lapp P, et al. The increased number of epithelial mast cells in nasal polyps and adjacent turbinates is not allergy-dependent. *Allergy* 1990;45:370-4.
27. Settiani GA, Chafee FH. Nasal polyps in asthma and rhinitis: a review of 6,037 patients. *J Allergy Clin Immunol* 1977;59:17-21.
28. Tos M, Mogensen C, Thompson J. Nasal polyps in cystic fibrosis. *J Laryngol Otol* 1977;91:827-35.
29. Stoop AE, van der Heijden HA, Biewenga J, Vandarbaan S. Eosinophils in nasal polyps in nasal mucosa: an immunohistochemical study. *J Allergy Clin Immunol* 1993;91:616-22.
30. Stoop AE, van der Heijden HA, Biewenga J, Vandarbaan S. Clinical aspects and distribution of immunologically active cells in the nasal mucosa of patients with nasal polyps after endoscopic sinus surgery and treatment with topical corticosteroids. *Eur Arch Otorhinolaryngol* 1992;249:313-7.
31. Bjerke K, Brandtzaeg P. Terminally differentiated human intestinal B cells. IgA and IgG subclass-producing immunocytes in the distal ileum, including Peyer's patches, compared with lymph nodes and palatine tonsils. *Scand J Immunol* 1990;32:61-7.
32. Brandtzaeg P. Immune functions and immunopathology of palatine and nasopharyngeal tonsils. In: Bernstein JM, Ogra PL, editors. *Immunology of the ear*. New York: Raven Press, 1987:63-106.
33. Hirata F, Schiffman E, Venkatasubramanian K, Axelrod J. Phospholipase A₂ inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proc Natl Acad Sci* 1979;76:2640-3.
34. English GM, Englewood CO. Nasal polypectomy and sinus surgery in patients with asthma and aspirin idiosyncrasy. *Laryngoscope* 1986;96:374-80.
35. Ogawa H. A possible role of aerodynamic factors in nasal polyp formation. *Acta Otolaryngol* 1986;430:18-20.