

Epithelial Shedding of the Inferior Turbinate in Perennial Allergic and Nonallergic Rhinitis

A Riddle to Solve

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Objective: To examine the epithelial integrity of the inferior turbinate in patients with perennial allergic rhinitis (PAR) and perennial nonallergic (vasomotor) rhinitis (PNAR).

Design: Nonrandomized, controlled morphometric study.

Setting: University-affiliated hospital.

Patients: Fifty-eight inferior turbinate samples were processed for histological study. Nineteen were from patients with PAR, and 20 were from patients with PNAR. Samples from 19 healthy individuals who underwent rhinoplasty for cosmetic reasons served as control specimens.

Main Outcome Measures: The length of the basement membrane (BM) covered with intact epithelium, covered with a single layer of basal cells, and devoid of epithelium was measured.

Results: Intact respiratory epithelium and areas of partial and complete epithelial denudation were encountered in control specimens and in samples from patients with PAR and PNAR. A significant difference was found between the 3 groups ($P=.001$). The proportion of the BM covered with undamaged epithelium was significantly greater in control specimens and in samples from patients with PNAR than in samples from patients with PAR; the difference between the former 2 groups was nonsignificant. Most of the epithelial damage in patients with PAR occurred between columnar and basal cells rather than between basal cells and the BM ($P=.02$).

Conclusions: Epithelial shedding of the inferior turbinate is a genuine feature of PAR and is not an artifact of tissue sampling. The finding of greater epithelial exfoliation between basal cells and the more superficial columnar cells than between basal cells and the BM probably reflects different attachment qualities of these cells.

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THE EPITHELIUM PROVIDES the first line of defense against invading microorganisms and foreign matter. One of the expressions of disrupted mucosal barrier function is epithelial shedding, which is considered part of mucosal remodeling in inflammatory diseases. Support for this long-recognized phenomenon can be found in qualitative observations of postmortem specimens and endobronchial biopsy specimens^{1,2} and in quantitative observations of the sputum and the product of bronchoalveolar lavage of patients with asthma.^{3,4} Epithelial shedding is attributed to the cytotoxic effects of inflammatory mediators such as major basic protein, eosinophilic cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin released from activated eosinophils.⁵ Ordóñez et al⁶ challenged this commonly accepted view,

arguing that epithelial denudation in asthma is not genuine but rather is an artifact of tissue sampling.

Investigators report that epithelial shedding also occurs in the nasal passages of patients with allergic rhinitis.⁷⁻¹⁰ Others not only failed to show a similar response in patients with allergic rhinitis but also questioned its existence.¹¹⁻¹⁴ Most of these observations were based on examination of tiny pieces of nasal mucosa using a light microscope or an electron microscope and were qualitative by nature. Among the few studies^{10,13,14} that performed actual measurements to assess the epithelial integrity of the inferior turbinate (IT) in patients with perennial allergic rhinitis (PAR), the study by Amin et al¹⁰ included patients with perennial nonallergic (vasomotor) rhinitis (PNAR).

The present study provides quantitative and qualitative data about the epithelial integrity of the IT in patients with PAR

and PNAR and compares these data with those of individuals who had no known history of nasal disease or allergy. Given the controversy surrounding this issue, our objective was to determine whether epithelial shedding of the IT in chronic hypertrophic rhinitis is a fact or an artifact.

METHODS

TISSUE COLLECTION

The material contained 58 samples of the IT. Thirty-nine samples were from patients who had prolonged nasal obstruction originating from marked bilateral IT hypertrophy; otherwise the patients were immunocompetent generally healthy individuals. The patients had failed to respond to medical treatment with antihistamines, decongestants, topical corticosteroids, and mast cell stabilizers given for at least 2 months before the decision was made to operate. Immunotherapy for the patients with a known history of nasal allergy had been ineffective or was refused. None received topical or systemic corticosteroids or had symptoms of upper respiratory tract infection during the month before surgery. Skin prick tests for 20 common airborne antigens were performed at the Allergy and Clinical Immunology Unit, Meir Medical Center, to differentiate between patients with and without allergy. Nineteen of 39 patients were allergic to house dust mite and were defined as having PAR. The remaining 20 patients had a negative response to the allergens and were defined as having PNAR. All patients underwent inferior turbinectomy that included removal of the bone and the surrounding mucosa.¹⁵ Nineteen individuals who had rhinoplasty for cosmetic reasons served as a control group. On examination, their IT appeared normal, and they had no symptoms or signs of nasal or other debilitating disease. Skin prick test results for 20 common airborne allergens were negative. To facilitate post-operative nasal breathing, the anterior third of the patients' IT was also excised during surgery. The three groups were age matched ($P = .15$). The study protocol was approved by the institutional review board of the Meir Medical Center, Kfar Saba. Known benefits, risks, complications, and alternatives to surgery were discussed preoperatively, and all patients signed an informed consent form before enrollment.

TISSUE PREPARATION

All samples underwent histological and immunohistochemical processing at the Ear, Nose, and Throat Histopathologic Research Laboratory of the Department of Otolaryngology–Head and Neck Surgery, Meir Medical Center. They were fixed in 10% buffered formaldehyde; thereafter, the IT bone was separated by sharp dissection. The samples were then dehydrated with increasing concentrations of alcohol and were embedded in paraffin blocks. Serial 5- μ m-thick tissue sections were cut at a plane perpendicular to the mucosal surface and were attached to slides coated with poly-L-lysine (Sigma-Diagnostics Inc, St Louis, Mo). Representative sections of the pathologic and normal ITs were stained with hematoxylin-eosin. The epithelium on mucosal surfaces, which is delicate and can easily break, was handled carefully to ensure the integrity of the samples at the time of retrieval and preparation.

MORPHOMETRIC MEASUREMENTS

The samples were coded at random and were analyzed blindly using an eyepiece of the microscope containing a 10 $\times$ 10-square reticule. The length of the reticule at magnification $\times$ 100

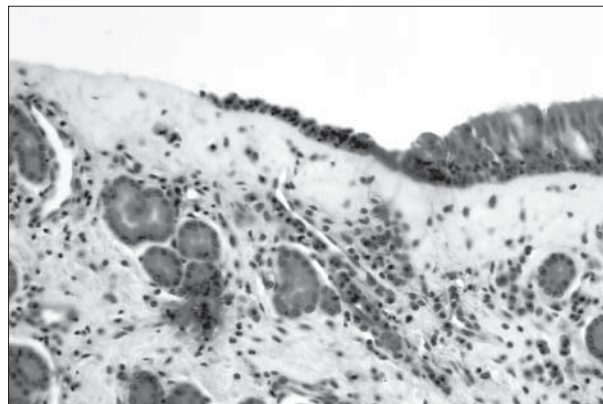


Figure 1. A section of the inferior turbinate from a patient with perennial nonallergic (vasomotor) rhinitis showing complete denudation (upper left) and partial denudation (upper middle) of epithelial cells; normal respiratory epithelium is shown on the upper right (hematoxylin-eosin, original magnification $\times$ 200).

was 0.8 mm. For accurate comparison, a section was selected from each sample, positioned at the same distance along the course of the anterior third of the IT and comprising the medial, inferior, and lateral surfaces. Similar to the example of Ordóñez et al,⁶ the length of the basement membrane (BM) (1) covered with intact pseudostratified ciliated columnar epithelium, (2) covered with a single layer of basal cells, and (3) devoid of epithelial cells was measured. The total length of the BM was determined by adding the lengths of the corresponding areas of each of the 3 measurements. The numerator for calculating the percentage (ie, the relative proportion) of the BM covered by each of these lengths was the sum of length of the corresponding areas, and the denominator was the total length of the BM.

Ethical reasons limit our ability to study all portions of the IT in healthy individuals; therefore, quantitative assessment was carried out for the anterior third of the IT in the controls. Qualitative assessment of the middle and posterior portions of the IT was performed only for patients with PAR and PNAR.

STATISTICAL ANALYSIS

The proportions were transformed to the arcsine square root scale to reduce differences in variance across groups and to improve the adequacy of the normal approximation. Analysis of variance using the Tukey method for multiple comparisons was performed to determine the epithelial integrity of the IT in patients with PAR and PNAR and in controls. Analysis of variance was also used for comparing epithelial length and age differences. The proportion of partial and complete epithelial denudation within each of the 3 groups was determined using the paired t test. Measurements were expressed as mean $\pm$ SD. Significance was set at $P < .05$.

RESULTS

Except for intact areas, the following 2 forms of epithelial damage were encountered: (1) partial denudation of epithelial cells (ie, the BM was covered with a single layer of basal cells without ciliated cells or goblet cells) and (2) complete denudation of epithelial cells, including basal cells. Both forms were observed in controls and in patients with PAR and PNAR but in varying proportions as noted herein (**Figure 1**).

Table. Epithelial Length and Relative Proportion of Intact, Partially Denuded, and Completely Denuded Basement Membrane in Patients With PAR and PNAR and in Control Subjects*

Group	Epithelial Length, mm†	Relative Proportion, %		
		Intact Epithelium	Partially Denuded Epithelium	Completely Denuded Epithelium
PAR	13.0 ± 5.2	42.7 ± 19.2	35.7 ± 14.6	21.7 ± 17.7
PNAR	10.9 ± 4.4	57.8 ± 22.6	26.3 ± 18.1	16.0 ± 11.2
Control	10.4 ± 4.7	72.1 ± 12.6	18.6 ± 10.0	9.2 ± 9.0

Abbreviations: PAR, perennial allergic rhinitis; PNAR, perennial nonallergic (vasomotor) rhinitis.

*Data are given as mean ± SD.

†As measured from the sections of the anterior one third of the inferior turbinate.

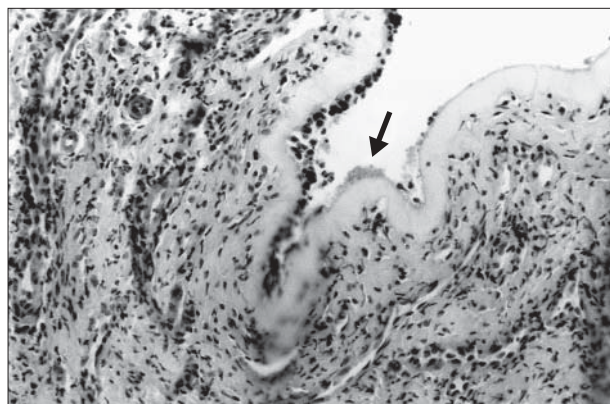


Figure 2. A section of the inferior turbinate from a patient with perennial allergic rhinitis showing a thin pinkish layer of plasma-derived gel (arrow) over the denuded basement membrane (hematoxylin-eosin, original magnification ×400).

QUANTITATIVE ASSESSMENT

As summarized in the **Table**, the mean lengths of the IT BM in patients with PAR and PNAR and in controls were similar (13.0 ± 5.2 , 10.9 ± 4.4 , and 10.4 ± 4.7 mm, respectively; $P = .20$). The Table also lists the relative proportions of intact partially denuded (ie, with a single layer of basal cells) and completely denuded BMs in patients with PAR and PNAR and in controls. Assessment of the epithelial integrity demonstrated a significant difference between the 3 groups ($P < .001$). Further analysis showed a significant difference between the relative proportions of the BM covered with undamaged epithelium in patients with PAR compared with those of controls and patients with PNAR. The difference between the latter 2 groups was insignificant. This indicates significantly greater epithelial damage in patients with PAR than in controls or in patients with PNAR. For the type of epithelial damage, a significantly greater percentage of the BM was covered with a single layer of basal cells compared with completely denuded areas in controls and patients with PAR ($P = .009$ and $P = .02$, respectively), whereas in patients with PNAR the difference did not reach statistical significance ($P = .12$). These findings attest to greater epithelial damage occurring between the basal cells and the more superficially columnar ciliated and goblet cells rather than between the BM and the epithelial basal cells.

QUALITATIVE ASSESSMENT

Qualitative assessment carried out for the middle and posterior portions of the IT in patients with PAR and PNAR showed the 3 stages of epithelial formation with marked partial and complete epithelial denudation. Examination of the completely denuded areas using high-power magnification revealed a thin pinkish layer of plasma-derived gel over the denuded BM (**Figure 2**) in some part of the samples from patients with PAR, probably reflecting the early stages of epithelial restitution.

COMMENT

There is debate as to whether and to what extent epithelial shedding occurs in allergic rhinitis. We found that the extent of epithelial shedding was significantly greater in patients with PAR compared with controls and patients with PNAR. This supports the hypothesis that allergic rhinitis is associated with increased epithelial damage and exfoliation of the IT. The inclusion of an appreciable number of samples for each of the 3 groups and the finding of epithelial shedding in healthy and inflammatory airways reduce the possibility of error and favor the conclusion that the phenomenon is genuine and is not an artifact of tissue sampling.

To the best of our knowledge, only 3 other studies have performed actual measurements to assess the epithelial integrity of the IT. One study¹⁰ agrees with our findings, and 2 studies^{13,14} failed to show significant differences between patients with PAR and a control group. We suspect that the disparity between the studies is attributable to differences in patient selection or in methods of investigation. Our study showed that epithelial shedding prevails in patients who had a relatively severe clinical course of nasal obstruction that was treated surgically after failed attempts with antihistamines, decongestants, topical corticosteroids, and mast cell stabilizers. Likewise, Amin et al¹⁰ provide laboratory evidence that epithelial shedding occurs in more severe cases of PAR, with greater sensitivity to pollens during the pollen season than out of season. The authors also observed a higher number of eosinophils in areas of epithelial exfoliation. Chanez et al¹³ did not find epithelial shedding in patients with asthma and PAR, who (according to their symptoms and endoscopic scores) had only mild to moderate severity of nasal disease. Contrary to our methods of investigation using representative sec-

tions of the medial, inferior, and lateral aspects of the anterior portion of the IT, both of the other studies^{13,14} used tiny biopsy specimens from the anterior end of the inferior aspect of the IT or from unspecified sites. Further investigations are warranted to examine whether epithelial damage occurs throughout the entire IT or whether it is confined to specific regions, as well as to assess the extent of the damage and whether it is homogeneously distributed along the various regions of the IT.

Although different in origin, PNAR shares common characteristics with PAR regarding mast cell and goblet cell populations.^{16,17} As mentioned earlier, patients with PNAR had an extent of epithelial shedding similar to that of controls but significantly less pronounced than that in patients with allergy. Others report the phenomenon in detail and found that (similar to patients with PAR outside of the pollen season) the BM of patients with PNAR displayed a significantly greater extent of epithelial denudation compared with controls but showed a lesser extent of denudation compared with patients with PAR during the pollen season.¹⁰

Partial epithelial denudation of the IT, which is characterized by a selective loss of columnar cells that leaves a single layer of basal cells to cover the BM, was significantly more abundant than complete denudation (ie, complete stripping of cellular coverage) in our patients with PAR. A similar pattern of epithelial exfoliation prevails in the lower airways of patients with asthma.¹⁸ In both locations, most epithelial disruption occurs in the "superficial plane" between the basal and columnar cells rather than in the "deep plane" between the BM and epithelial basal cells. This may be explained by differences in anchoring characteristics of columnar and basal cells. It seems that the anchoring of the columnar cells to basal cells through desmosomal connections¹⁹ is less resistant and is easily disrupted compared with the anchoring of the basal cells to the lamina densa of the BM, where the attachment is through hemidesmosomal connections and other adhesive mechanisms involving integrins and anchoring filaments.²⁰

Bronchial epithelial disruption of patients with asthma is traditionally related to cytotoxic effects of eosinophil granule proteins. A similar mechanism was shown in the paranasal epithelium of patients with chronic sinusitis.²¹ The following 2 other mechanisms inducing epithelial damage were suggested: (1) plasma exudate leaking through vascular epithelial gaps and (2) disturbances in cell-to-cell adhesion.¹³ Regarding disturbances in cell-to-cell adhesion, the authors showed that higher expression of the anti-adhesion molecule episialin, which inhibited integrin-mediated cell adhesion to extracellular matrix components and caused marked reduction in cell adhesiveness, was found in the bronchial epithelium of patients with asthma. Given that the mechanism underlying epithelial shedding in the IT of patients with PAR is not fully understood, further study is needed to establish whether these mechanisms or others apply.

Experimental tracheal deepithelialization induced rapid formation of a protective extravasated plasma layer over the BM minutes after denudation, followed by epithelial cell dedifferentiation and migration to fill

the gap.²² In our study, we observed a thin pinkish gel layer over the denuded BM in patients with PAR. The gel is suggested to be of plasma-derived factors and probably reflects an early phase of epithelial restitution.²² This finding further supports our conclusion that epithelial shedding is a genuine pathologic expression of disrupted mucosal barrier function in patients with PAR.

CONCLUSIONS

The present study demonstrates a significantly greater proportion of epithelial shedding in patients with PAR compared with controls and patients with PNAR, suggesting that the phenomenon is a genuine feature and is not an artifact of tissue sampling. The greater epithelial damage found between basal cells and the more superficial columnar cells than between basal cells and the BM probably reflects the existence of different attachment qualities of these cells.

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Author Contributions: Drs Berger and Ophir had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Berger. *Acquisition of data:* Berger. *Analysis and interpretation of data:* Berger, Bernheim, and Ophir. *Drafting of the manuscript:* Berger. *Critical revision of the manuscript for important intellectual content:* Berger, Bernheim, and Ophir. *Obtained funding:* Ophir. *Administrative, technical, and material support:* Berger. *Study supervision:* Berger, Bernheim, and Ophir.

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