Histology of nasal mucosa in normals and in patients with perennial rhinitis

A blind study of plastic embedded specimens

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SUMMARY

A blind study of the histology of nasal mucosa in normals and in patients with perennial rhinitis was performed. Specimens were embedded in plastic and sections were examined by light and anoptral contrast microscopy. In an analysis of 18 measured variables the following were the major differences found between the two groups. The normals had the highest percentage of squamous epithelium. Many goblet cells were seen in patients with perennial rhinitis, especially those with pronounced nasal obstruction. Patients with perennial rhinitis had by far the highest number of eosinophils in the submucosa and especially in the epithelium.

INTRODUCTION

In the last decade the ultrastructure of the nasal mucosa from normals and from patients with allergic diseases has been studied by transmission and scanning electron microscopy (Jahnke, 1972 b, Lenz, 1973, Mygind and Bretlau, 1973). For several decades quantitative investigations have been carried out, using paraffin-embedded specimens (van Dishoeck and Majer, 1964). Quantitative studies of the allergic mucosa have however not been done using semithin sections of plastic-embedded specimens, which allows a better identification of different cell-types and cellular structures than with paraffin-embedded specimens. It is now customary for clinical and quantitative histological investigations to be carried out on a blind basis in order to exclude bias. If an investigater anticipates his findings there is a natural tendency for him to obtain results similar to previous investigations and thus merely confirm previous work.

In the following we present the results of a blind histological investigation of plastic-embedded biopsies of the nasal mucosa from patients with perennial rhinitis and from normal volunteers.

METHODS AND MATERIAL

A study of the influence of disodium cromoglycate and preservatives on the nasal mucosa in normal volunteers and in patients with perennial rhinitis has

been performed (Mygind et al., 1974). The present investigation is part of that study and constitutes the pre-treatment histology of the nasal mucosa.

16 volunteers without allergic diseases (mean age: 24 years) and 16 patients suffering from perennial rhinitis (mean age: 31) were entered into the study. A careful case history was taken which included symptoms assessed by daily diary cards, allergological examination, blood tests and nasal smears in all patients. A biopsy specimen (1½—3 mm) was taken with a small pair of forceps without local anaesthesia from the lower edge of the inferior turbinate ½—1 cm behind the front edge. The biopsy was immediately fixed in cold 5 per cent glutaraldehyde, buffered with a 0.03 M sodiumcacodylate (pH: 7.4). After 24 hours fixation the biopsy was stored in 0.15 M sodiumcacodylate (pH: 7.4). After dehydration in ethanol the specimens were embedded in Epon, and 2 μm thick sections were cut in a Reichert ultramicrotome UM 2 with a Dupont diamond knife.

Each biopsy provided 1—5 Epon blocks. All blocks were cut, and either one or two of the best specimens were investigated. Four serial sections were stained by 1 per cent toluidine blue for normal light microscopy and four remained unstained for examination in anoptral contrast. The blocks were cut down and serial sections from another part of the specimen were treated in a semilar manner. The sections were coded, and 18 different variables were measured blindly (Table I). The mean for each measurement was calculated for each subject and for the two groups.

Table 1. The eighteen histological measures made, and their units of measurements

Histological variable	Unit of measurement
Smooth epithelium Uneven epithelium Pseudostratified columnar epithelium	percentage of each
Intermediate epithelium Squamous epithelium	percentage of each
Cilliated cells	number per high power field
Goblet cells	number per high power field
Basement membrane thickness	μm
Eosinophil leucocytes in epithelium	number per high power field
Neutrophil leucocytes in epithelium	number per high power field
Mast cells in epithelium	number per high power field
Eosinophil leucocytes in submucosa	number per high power field
Neutrophil leucocytes in submucosa	number per high power field
Mast cells in submucosa	number per high power field
Plasma cells in submucosa	number per high power field
Mononuclear roundcells in submucosa	number per high power field
Apocrine secretion	0, +, ++, +++
Intercellular oedema	0, +, ++, +++

Tables II. T-test of baseline histological data, comparing subject types

Variable	Means for: Volunteers	Patients	T-value	d.f.	2-tail Significance
% smooth epithelium	66,42	59.33	-0.118	29	P>.05
% uneven epithelium	33,58	40.67	-1.263	29	P>.05
% pseudostratified columnar epithelium	27,50	68.33	-3.119	29	P<.01
% intermediate epithelium	55,36	31.67	1.126	29	P>.05
% squamous epithelium	17,14	0,00	1.883	29	P<.05
No. of cells with cillia per h.p.f.	0.08	2.78	2.555	*14	P<.05
No. of globlet cells per h.p.f.	0.95	4.12	3.469	*23	P<.01
Thickness of basement membrance, μm.	8.12	10.33	2.002	29	P>.05
Eosinophil leucocytes in epithelium	0.01	0.25	2.702	*14	P<.05
Neutrophil leucocytes in epithelium	0.51	0.20	1.712	*21	P>.05
Mast cells in epithelium	0.02	0.13	2.053	*15	P>.05
Eosinophil leucocytes in submucosa	0.17	1.06	2.644	*16	P<.05
Neutrophil leucocytes in submucosa	1.48	0.97	1.449	*22	P>.05
Mast cells in submucosa	1.05	0.99	0.218	30	P>.05
Plasma cells in submucosa	1.20	0.65	0.751	*17	P>.05
Mononuclear roundcells in submucosa	2.60	1.81	1.682	30	P>05

RESULTS

The number of histological sections cut and examined was very large thus it is only possible to present the mean values for the measurements in the two groups, normals and patients (Table II).

In the epithelial surface (smooth/uneven) no differences were seen between the two groups; however, most of these small biopsies were cut obliquly which makes the interpretation of the results difficult and somewhat uncertain.

The normals had more squamous epithelium than the patients; the opposite was the case with the pseudostratified columnar epithelium and this difference was significant ($P<0.01$). In normals and in patients intermediate epithelium was seen which was neither typical squamous nor typical pseudostratified.

One of the most striking phenomena noted was in the goblet cells; 12 out of 15 patients had goblet cells in the epithelium, the mean count per high power field was 4.1; only 5 healthy subjects showed this cell and the mean was 0.95. Perusal of the clinical diary cards showed that in 7 patients a major clinical symptom was nasal obstruction; in these patients the mean goblet cell count was 6.7, whereas in the remaining patients it was 2.4.

*) Concerning the so-called apocrine secretion (cytoplasm protuberances) from the epithelial cells and concerning intercellular oedema no differences between the two groups were observed.

No difference in the thickness of the basement membrane (connective tissue membrane) could be demonstrated; again the oblique cutting made the interpretation of the result difficult.

Neutrophils mononuclear roundcells and plasma cells were studied; all three were usually present in volunteers and patients and there was no difference between the two groups. Neutrophils were found in the bloodvessels, the submucosa, the epithelium and in the secretions; more neutrophils were seen between blood-vessels and the epithelium than elsewhere in the submucosa indicating the existence of chemotactic factor in their secretion. The cells identified as mononuclear roundcells consisted of lymphocytes, transverse sectioned fibrocytes or other connective tissue cells. Only in the transmission electron microscope is it possible to state the exact number of lymphocytes alone; they were not observed in the epithelium, which accounts for the lack of this cell in the nasal smear. Plasma cells were generally scarce but they occured constantly; in a few specimens large numbers of plasma cells were seen so that this was the most frequent cell type in the submucosa.

Mast cells were found in all biopsies without quantitative or apparently qualitative differences between normals and patients; but there was a slightly increased number of these cells in the epithelium of patients.

The presence of eosinophils in the epithelial layer was seen in 11 patients but in only two volunteers; in the submucosa they were seen in 13 patients and 11 volunteers. The number present in the volunteers was low (mean 0.17) compared to patients (mean 1.1.). In three patients eosinophils were not seen in either the submucosa or the epithelium, in two they were absent in the nasal smears; as the diary cards of these patients only showed minor symptomatology, it is questionable whether they should be considered to be suffering from perennial rhinitis.

DISCUSSION

A comparison of the histological data of patients and volunteers revealed some unexpected results. Patients had significantly more pseudostratified columnar epithelium and ciliated cells in the anterior part of the nasal cavity than normal volunteers; the reverse might have been expected. It is considered that this finding can be explained by the following; the anterior third of the mucosa in the nasal cavity in normal persons undergoes total, or partial metaplasia to a squamous or intermediate epithelium due to the continuous influence of the normal inspired air; if this influence ceases due to nasal obstruction or laryngectomy (Hilding and Hilding, 1970; Jahnke, 1972 b), the epithelium reverts to a pseudostratified columner epithelium. This may be the case in patients with perennial rhinitis who have nasal obstruction and thus breathe through the mouth.

Patients had significantly more goblet cells than volunteers; this finding is in agreement with other investigators. A report of an extensive investigation of nasal smears (Bryan and Bryan, 1959) showed that the significant cells of nasal secretion in allergic rhinitis were goblet cells, eosinophils and mast cells; however,

in a large group of children (Murray and Anderson, 1969) no significant association could be found between clinical evidence of nasal allergy and goblet cells in nasal secretion. The latter finding could be due to the fact that in children goblet cells are not easily exfoliated when obtaining nasal smears because the mucosa is wiped somewhat gently in order to minimise the inconvenience to the young patient. The fact that we found a significant increase in goblet cells in adult patients may partly be due to the decreased amount of squamous epithelium and partly to an actual increase of goblet cells in the pseudostratified epithelium. The fact that patients with pronounced nasal obstruction had the largest number of globet cells explains the common clinical observation of a viscid nasal secretion in these cases. The same mechanism, i.e. the cessative of the continuous influence of the inspired air may explain why the volume of sputum in patients with bronchial obstruction (asthma, neoplasm) is sometimes excessive.

The examination of neutrophils, plasma cells and mononuclear roundcells did not disclose any evidence of type III or type IV reactions (Arthus reaction, cell-mediated hypersensitivity). The number of plasma cells in the submucosa were constant and low; but in a few patients a large number were seen resembling the distribution in nasal polyps. No plasma cells were observed in the epithelium, in contrast to the neutrophils, which apparently wander directly from the blood-vessels through the submucosa and the epithelium to the secretion, probably attracted by chemotaxis from microbial products.

The number of mast cells is variable; in acute allergic reactions (type I reactions) few will be seen, however, in chronic inflammatory reactions there will be an increase. We found no difference in mast cells between patients and volunteers which suggests that in perennial rhinitis there is an equilibrium between acute allergic reactions and chronic inflammatory processes. Patients had an insignificantly higher number of mast cells in the epithelium than normals; however we think that this reflects a real difference as earlier studies (Bryan and Bryan, 1959; Mygind and Thomsen, 1973) have demonstrated a definite increase in the number of mast cells in nasal smears from patients with perennial rhinitis. It is uncertain whether this reflects a chemotatic influence on the submucosal mast cells, or whether it is due to an overall increase in the permeability of the epithelium.

Eosinophilia in the mucosa was a constant phenomenon in patients with perennial rhinitis, irrespective of whether they had a positive or negative allergic examination. The difference between normal volunteers and patients was much more distinct with regard to eosinophils in the epithelial layer than for those in the submucosa; this is in accordance with the observation that an eosinophilia is much more pronounced in the secretions than in the mucosa. It could be that some factor in the secretion is "calling for the eosinophil cell"; as rhinitics have an increased number of mast cells in the secretion this could be the eosinophil chemotactic factor of anaphylaxis; however there is still a great lack of knowledge concerning the possible function of mast cells in the epithelial layer and in the secretions.

ZUSAMMENFASSUNG

Es wurde eine blinde Untersuchung über die Histologie der Nasenschleimhaut bei Normalen und bei Patienten mit vasomotorischer Rhinitis unternommen. Die Präparate wurden in Plastik eingelagert und die Schnitte im Lichtmikroskop und anoptral Kontrastmikroskop untersucht. In einer Analyse von 18 Parametern wurden die folgenden Hauptverschiedenheiten zwischen den beiden Gruppen festgestellt. Die Normalen hatten prozentisch den grössten Anteil von Pflasterepithel. Bei den Patienten mit vasomotorischer Rhinitis, und besonders bei denen, die von ausgesprochener Verlegung der Nasenatmung litten, wurden viele Becherzellen gefunden. Die Patienten mit vasomotorischer Rhinitis hatten bei weitem die grösste Anzahl von Eosinophilen in der Submukosa und im Epithel.

REFERENCES

1. Bryan, W. T. K. and Bryan, M. P., 1959: Cytologic diagnosis in otolaryngology. Trans. amer. Acad. Ophtal. Otolaryng., 63, 597-612.
2. van Dishoeck, H. A. E. and Majer, E. H. in Berendes, J., Link, R. and Zöllner, F., 1964: Hals-Nasen-Ohrenheilkunde. Vol. I, 365-412. George Thieme, Stuttgart.
3. Hilding, D. A. and Hilding, A. C., 1970: Electron microscopic observations of nasal epithelium after experimental alteration of air flow. Ann. Otol-Rhinol. Laryng., St. Louis, 79, 451-460.
4. Jahnke, V., 1972a: Ultrastruktur der Nasenschleimhaut nach Larynctomie. Arch. Ohr-, Nas.- u. KehlkHeilk.
5. Jahnke, V., 1972b: Ultrastruktur der allergischen Nasenschleimhaut des Menschen. Z. Laryng. Rhinol., 51, 152-162.
6. Lenz, H., 1973: Three-dimensional surface representation of the cilia-free nasal mucosa of man. Acta oto-laryng., Stockh., 76, 47-57.
7. Murray, A. B. and Anderson, D. O., 1969: The epidemiologic relationship of clinical nasal allergy to eosinophils and goblet cells in the nasal smear. J. Allergy, 43, 1-8.
8. Mygind, N. and Bretlau, P., 1973: Scanning electron microscopic studies of the human nasal mucosa in normals and in patients with perennial rhinitis. I: cilia and microvilli. Acta allerg. (Kbh.), 28, 1-27.
9. Mygind, N. and Thomsen, J., 1973: Cytology of the nasal mucosa. A comparative study between a replica-method and a smear-method. Arch. klin. exp. Ohr.-, Nas.- u. Kehlk.Heilk.
10. Mygind, N. Viner, A. S. and Jackman, N., 1974: The influence on nasal mucosa of unpreserved and preserved nasal sprays containing disodium cromoglycate. Rhinology, 12, 49-54.

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