Rhinology, 44, 188-192, 2006

The influence of air current on goblet cell density in the mucosa of the normal uncinate process in the nasal cavity*

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SUMMARY

Aim: *To determine the density of goblet cells in the mucosa at the medial site of the uncinate process in the nasal cavity where the air current is high and compare with the goblet cell density on the protected lateral site of the uncinate process, thus establishing the function of the uncinate process in protecting the integrity of epithelium, in the meatus medius, from the distress caused by continuous air flow. Determining this function could have clinical implications, as patients routinely undergo removal of the uncinate process during endoscopic sinus surgery.*

Material and methods: *The material comprised pieces of mucosa of 11 uncinate processes from the nasal cavity of 11 autopsies. The material was stained with PAS-Alcian blue after which the goblet cells were counted using a whole-mount method. Counting was done by only 1 of the authors and was not blinded.*

Results: *The density of goblet cells was significantly higher on the lateral site than on the medial site of the uncinate process. Furthermore, goblet cell density was found to be higher on the superior part of the uncinate process, than on the inferior part.*

Conclusion: *The results seem to indicate that air current has consequential impact on the normal differentiation patterns of the epithelium in the nose. The significantly higher goblet cell density on the unexposed lateral site of the uncinate process suggests that at this site the sinus outlets are protected from air current trauma.*

Key words: air, goblet cell density, mucociliary clearance, middle meatus, ostiomeatal complex, PAS-Alcian blue whole-mount method

INTRODUCTION

Goblet cells are mucus-producing elements of the respiratory airway epithelium and play an important role in muco-ciliary clearance and in the local mucosal immune system. Quantitative studies have substantiated that goblet cell density varies in different parts of the respiratory tract and is influenced by various factors such as genetic factors, a combination of hereditary factors and environmental factors during early infancy, the impact of air current, as well as infection or inflammation [(1)].

We have chosen to single out the impact of air current as a determinant of goblet cell density at the exposed medial site, compared with the density at the protected lateral site. The shape and location of the uncinate process (UP) at the anterior part of the nasal middle meatus indicates that its main function is to protect the important middle meatus epithelium from trauma caused by the continuous flow of air through the nasal cavity. The prominent medial site of the UP is exposed to the air current, while the mucosa at the lateral site is hidden by and therefore not exposed to the same degree of trauma. Accordingly, the working hypothesis of this study was the expectation of a higher goblet cell density on the protected lateral site than on the exposed medial site of the UP. A positive result could help to establish the protective function of the UP to air current trauma of the epithelium in the ostio-meatal complex.

As far as could be ascertained, there are no previously published studies on goblet cell density of the normal UP epithelium or on the epithelium of the middle meatus, but in earlier studies of the normal nasal septum and nasal turbinates done in our laboratory by Mogensen and Tos [(2)], it was shown that air current had a consequential influence on goblet cell density. In the normal adult nasal epithelium of 10 septums obtained from autopsy, consisting of 12 localities sectioned

*Received for publication: February 10, 2005; accepted: December 12, 2005

into 10 counting fields of 0.01768 mm^2 and thus giving 120 countings per septum, these authors found a significant increase in median goblet cell density in the anteroposterior direction, being 4800 goblet cells per square mm in the anterior and 6200 in the posterior quarter of the septum.

In another study of normal adult nasal turbinates, also by Mogensen and Tos [3], a highly significant anteroposterior increase of median goblet cell density was found in the medial and lateral aspects of the middle turbinate, but only in the medial aspect of the inferior turbinate. In the lateral aspect of the inferior turbinate, the density was found to be relatively high in the anterior part, in accordance with the relatively weak air current in the inferior meatus.

Wake et al., [4] described histological features of the UP mucosa removed from 16 patients undergoing endoscopic sinus surgery for chronic sinusitis. Their study demonstrated a relatively high proportion of goblet cells in the ciliary pseudostratified cylindrical epithelium and seromucous glands of the lamina propria. With scanning electron microscopy, four categories of surface morphology were noted in the epithelium:

1) The most common appearance was a predominance of ciliated cells (10 patients);
2) goblet cell dominance (3 patients);
3) nonciliated cell dominance (2 patients);
4) squamous cell dominant epithelium, associated with a long history of sinonasal symptoms and a higher incidence of surgical interventions (1 patient).

A similar ultrastructure of UP epithelium in chronic sinusitis, with damage of cilia and presence of squamous cell metaplasia, was described by Huang et al. [5].

Several authors have described variations of surgical anatomy of the UP [6,7].

MATERIAL AND METHODS

Study material

The material comprised pieces of mucosa of 11 UP′s from 11 autopsies (median age at time of death 74 (55-94) years). The causes of death, confirmed at autopsy, were: acute myocardial infarction (3), lung cancer (2), acute abdominal disease (2), pneumonia (1), ruptured aorta aneurysm (1), endocarditis (1), unknown cause (2). None had exhibited signs of acute or chronic nasal or paranasal sinus infection around the time of death. It was known that one patient (No 14) had been allergic to cats and acetylsalicylic acid. There was no information on the other patients concerning possible allergies.

As a part of our autopsy studies on the origin of nasal polyps [8,9], the nasal cavity and the sinuses were examined with a 30 degree (angle) sinus endoscope in all subjects. If nasal polyps or signs of active sinusitis were found, the patients were excluded from the study. If no pathology was found a biopsy was taken and examined.

To examine the pieces of mucosa, a whole mount method [10] where, after fixation with formol alcohol, entire mucosa pieces were fine-dissected under the stereomicroscope, removing the deeper layer of the lamina propria with the deep gland layer, the vascular plexus and the superficial gland layer. This fine dissection is difficult and time-consuming, but necessary so that the mucosa is so thin that it consists only of the epithelium with the basement membrane and a very thin layer of lam-

Figure 1. Whole-mount specimen stained with PAS-Alcian bue and placed in a chamber filled with aniseoil- colophonium, sealed with paraffin. The specimen was taken from the supero-medial aspect of the uncinate process.

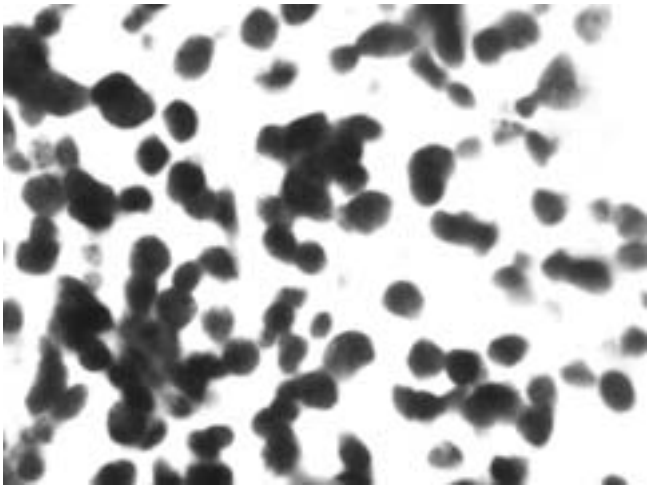

Figure 2. High density of goblet cell found on the lateral, protected side of the uncinate process. PAS-Alcian blue whole mount specimen, x 500.

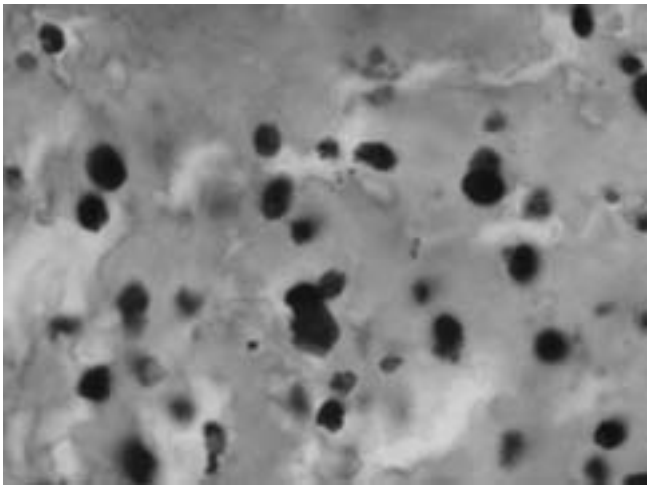

Figure 3. Low goblet cell density found on the medial, exposed side of the uncinate process. PAS-Alcian blue whole mount specimen, x 500.

ina propria without glands and capillaries.

The thin mucosa pieces were stained with PAS-Alcian blue and cleared in anise oil [10]. The mucosa pieces were placed in separate chambers that were filled with anise oil colophonium[11] and sealed with paraffin (Figure 1).

Two localities were investigated, a superior and an inferior on the medial side of the UP and two similar localities on the lateral side of the UP. In each locality goblet cells were counted in 10 counting fields of 0.01768 mm^2. Counting was performed by one of the authors and was not blinded, using Reichardt´s projection microscope (Visopan) with a magnification of x 500. Because of laceration and trauma of the epithelium during the removal or during micro-dissection of the mucosa on the lateral side of the UP, a comprehensive count could be performed in only 33 localities with 330 counting fields.

Statistics

Only one person was counting the cells and therefore there were no intraobserver variation. As mentioned above the counting was not blinded out of technical reasons. Due to a relative small amount of data, the Wilcoxon´s test for paired data was used. The data was controlled and recalculated by a statistician of the Institute for Biostatistic at the University of Copenhagen.

RESULTS

Morphology and distribution

In the whole mounts, goblet cells appeared as round to oval, sharply defined blue patches – thecae - on a pale background (Figures 2 and 3). The diameter was in most cases 5-10 μ, in a few less, 2-3 μ, or more, 11-15 μ. Large and small thecae were observed in all localities.

The goblet cells occurred in clusters of 2-6 cells or isolated (Figures 2 and 3) and were present in all counting fields. Their distribution was irregular, and present over a relatively wide range within the counting fields (Table 1).

Density in the medial and lateral wall

In the superior half of the **medial** (exposed) side of the UP epithelium, the inter-individual goblet cell density was 100 cells/field (Table 1), corresponding to 5656 cells per mm^2. The density was significantly higher in the superior part (Wilcoxon`s test $p < 0.02$) than in the inferior part with only 73 cells / field, corresponding to 4129 cells / mm^2. The inter-individual median density of the entire medial wall was 84 cells / field, corresponding to 4751 cells/mm^2.

In the **lateral** (protected) site, the inter-individual median goblet cell density was 111 cells / field (Table 1), corresponding to 6278 cells / mm^2, which is a significantly higher density than in the exposed mucosa from the medial site (Wilcoxon's test, $p<0.03$).

DISCUSSION

Quantitative studies showed a significantly lower density of goblet cells in the epithelium of the exposed medial site of the UP than on the protected lateral site. Furthermore, a signifi-

Table 1. Density of goblet cells found on the medial and lateral aspect of the uncinate process. Each side further divided in superior and inferior halves. Range values in ( ).

autopsy	age		medial site			lateral site		
number	years	sex	superior	inferior	both	superior	inferior	both
14	55	F	108 *(78-143)*	96 *(80-116)*	100 *(77-143)*			
16	89	M	108 *(65-136)*	116 *(63-139)*	110 *(63-139)*	108 *(88-131)*	123 *(92-179)*	114 *(88-179)*
17	82	F	87 *(48-115)*	93 *(55-130)*	87 *(48-130)*		143 *(68-190)*	143 *(68-190)*
18	62	M	55 *(38-72)*		55 *(38-72)*	103 *(60-134)*		103 *(60-134)*
136	65	M	84 *(39-103)*	84 *(70-102)*	84 *(39-103)*	150 *(108-197)*	109 *(86-122)*	121 *(86-197)*
137	68	M	100 *(70-112)*	69 *(50-99)*	84 *(50-112)*			
138	94	F	125 *(95-150)*	55 *(13-90)*	93 *(13-150)*			
139	82	M	102 *(75-118)*	74 *(52-125)*	83 *(52-125)*	88 *(77-104)*		88 *(77-104)*
141	71	M	68 *(51-83)*	40 *(26-87)*	55 *(26-87)*			
142	69	F	57 *(39-80)*	25 *(9-30)*	35 *(9-80)*	113 *(109-146)*	95 *(62-113)*	108 *(62-146)*
143	69	M	103 *(62-119)*	71 *(24-110)*	87 *(24-119)*		122 *(105-148)*	122 *(105-148)*
interindividual median			100	73	84	108	122	111
range			*(55-125)*	*(25-116)*	*(35-110)*	*(88-150)*	*(95-143)*	*(88-143)*

cantly lower density was found in the inferior part of the medial site of the UP than in the superior part. Epithelium of the inferior part of the UP borders onto the medial site of the inferior turbinate, where the goblet cell density is low and a transformed squamous epithelium is often found, and is presumably caused by the air current [3].

Several other findings indicate that the low density of goblet cells on the medial site of the UP is a consequence of the constant effect of air current to the cylindrical respiratory epithelium of the prominent and exposed medial site of the UP: scanning electron microscopy studies have shown damage to the UP cilia with squamous cell metaplasia [4,5]. Quantitative studies have demonstrated a significant anteroposterior increase of goblet cell density on the nasal septum and turbinates [2,3], while scanning electron microscopic studies have shown anterior-posterior increase of ciliated cells in the human nasal epithelium [12].

A strong argument for air current distress of the epithelium is the difference in goblet cell density in the anterior third of the inferior turbinate. On the medial site of the inferior tubinate, the median density was 117 cells / field, or 6617 cells / mm^2, while on the corresponding lateral site the density was 201 cells / field or 11368 cells / mm^2 [3]. In the epithelium of the medial site of the UP the interindividual density was 84 cells / field or 4751 cells / mm, and on the lateral site, 111 cells / field, or 6278 / mm^2 (Table 1). The lateral wall of the inferior turbinate is hardly visible, even by endoscope, as is the lateral wall of the UP, and there is presumably no air current trauma to these two walls.

Even though there are no calculations on the impact of air current on the various areas of the human nasal epithelium, and consequently no direct proof that the epithelial changes are a natural variation due to constant air flow through the nose, the demonstrated changes in goblet cell density indirectly indicate that this is so.

Our previous work on goblet cell density in normal human nasal epithelium, including this work on UP epithelium, strongly indicates that decrease of density expresses distress of the epithelium by air current, while increase of goblet cell density expresses no air current distress.

Other experimental studies provide further support for these statements. In Hilding's pioneer experimental work [13] in which he closed one nostril in rabbits, Hilding demonstrated that the nasal epithelium is transformed into a thick, stratified epithelium on the open side when air flow is doubled. On the surgically closed side, considerable increase of goblet cells and reduction of ciliated cells was found. In a subsequent study [14], however, a pronounced ciliogenesis was found.

Tos and Mogensen [15] repeated and further extended these experiments by surgical closure of one nostril of 20 rabbits for 4-90 days and performance of blinded quantitative histological study. **Posteriorly** on the septum on histological sections, they did not find any difference between the closed and open site, even after 90 days of closure. **Anteriorly**, however, the changes of the epithelium were massive, passing from damage of ciliary cells and vascular dilatation on the 4^{th} day, to hyperplasia of the basal cells and desquamation of the ciliated and goblet cells on the the 6^{th} day, at which time the epithelium had an appearance of thickened squamous epithelium. On the 10^{th} day, the round basal cells had changed into columnar cells and had begun to differentiate into ciliated cells and goblet cells, so that at the 16^{th} day the epithelium was, again, seen as columnar pseudostratified and ciliated. Tos and Mogensen [15] showed that continued doubled air current, again, is the cause of epithelial trauma, with injury to the ciliated cells and new cycles of repair with hyperplasia of basal cells and differentiation. Such changes were found on the 30^{th}, 60^{th} and 90^{th} day, but the following cycles of repair were not so drastic as the first. These experimental studies have shown that respiratory tract epithelium exposed to strong air current is damaged and in a constant state of repair [16].

Interestingly, there was increased secretory activity on the closed side with no airflow. On the 60^{th} and 90^{th} days the epithelium appeared on the closed side as a regular respiratory epithelium with many goblet and ciliated cells that extended right to the skin.

In Mogensen and Tos' study [17], they also demonstrated that the density of goblet cells on the protected side was significantly higher than on the open side.

It can be concluded that lower goblet cell density in the epithelium of the medial site of the UP is a consequence of the impact of the air current. The UP presumably protects the epithelium of the ostio-meatal complex from air current trauma, but we do not as yet know the degree of the epithelial changes after removal of the UP during endoscopic sinus surgery. More research on the muco-ciliary clearance and on morphological changes of the epithelium of the middle nasal meatus before and after removal of the UP is necessary.

ACKNOWLEDGEMENTS

We want to thank FoQus, Hillerød County Hospital, for financial support of this project, Mrs. Susanne Ditlevsen and the Institute for Biostatistic at the University of Copenhagen.

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