

ORIGINAL PAPER

Mucin genes have different expression patterns in healthy and diseased upper airway mucosa

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Clinical and Experimental Allergy

Summary

Background Mucus hyper-secretion is a feature of several airways diseases such as chronic rhinosinusitis, asthma, and cystic fibrosis (CF). Since mucins are major components of mucus, the knowledge of their distribution and regulation in nasal tissues is likely to improve mucus hyper-secretion therapy.

Objective The aim of this study was to evaluate and compare mucin gene expression at epithelial and glandular levels, and to identify potential mucin expression patterns for specific upper airways pathologies.

Methods Immunohistochemistry for MUC1, MUC2, and MUC4–MUC8 mucins was performed on healthy nasal mucosa (NM; $n = 12$), bilateral nasal polyps (NP; $n = 38$), NP from CF patients ($n = 10$), and antrochoanal (AC) polyps ($n = 11$). MUC2, MUC4, MUC5AC, and MUC6 mRNA expression were also analysed by *in situ* hybridization.

Results MUC1, MUC4, and MUC5AC mucins were highly expressed in the epithelium and their expression pattern was similar in all NP types, MUC1 and MUC4 being increased and MUC5AC decreased compared with NM. MUC8 was highly detected at both epithelial and glandular levels with marked variability between groups. MUC5B was mainly detected in glands and the expression in all polyp types was higher than in NM. Moreover, MUC5B expression was higher in NP epithelia from CF patients than in bilateral NP and healthy NM. Although MUC2 expression was low, especially in AC polyps, it was detected in most samples. In NM, MUC6 and MUC7 were scarcely detected and MUC7 expression was restricted to glands.

Conclusions These results suggest that NP have a different pattern of mucin expression than healthy NM and that CF polyps (increased MUC5B) and AC polyps (decreased MUC2) have a different mucin expression pattern than bilateral NP.

Keywords airways diseases, hyper-secretion, mucins, nasal mucosa

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Introduction

Mucus is the layer that covers, protects, and lubricates the luminal surfaces of epithelial respiratory, gastrointestinal, and reproductive tracts. Airway mucus is composed of water, ions, lung secretions, serum protein transudates, anti-microbial proteins, and mucus glycoproteins (mucins). The viscoelastic properties of mucus are mainly determined by the presence of mucins [1] that are high-molecular weight proteins extensively glycosylated, synthesized, and secreted by epithelial goblet cells and submucosal glands [2].

Genes encoding for 18 human apomucins (mucin protein backbones) have been cloned [3, 4]. Although eight of them, MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC7, MUC8, and MUC13 [5, 6], are normally expressed in the human respiratory tract, only MUC5AC and MUC5B have been convincingly demonstrated to be major components of human airway secretion [7, 8]. There are two major classes of mucins: secreted mucins (MUC2, MUC5AC, MUC5B, MUC6–MUC10, and MUC13–MUC16, MUC19) and membrane-bound mucins (MUC1, MUC3, MUC4, MUC11, MUC12, MUC17), which contain a transmembrane domain and a short cytoplasmic tail. From the

secreted mucins, *MUC2*, *MUC5AC*, *MUC5B*, and *MUC6* genes, are clustered in the 11p15 chromosomal region [9], and contain cysteine-rich domains homologous to von Willebrand factor D domains that are oligomerization sites. A common feature of both kinds of mucins is the presence of tandem repeats rich in Thr, Ser, and Pro, where the *O*-glycosylation occurs [10].

In chronic inflammatory airway diseases such as asthma, chronic bronchitis, chronic rhinosinusitis and nasal polyposis, and cystic fibrosis (CF), submucosal glands become enlarged, and the number of goblet cells increases in the airway surface epithelium, appearing in the distal airways where they are not normally present [11]. These cellular changes often result in mucus hyper-secretion usually with altered physiological properties. Different studies have demonstrated that mucus-secreting cells of nasal polyp (NP) epithelium contribute to increase mucus secretion [12, 13]. Bronchial goblet cells hyperplasia also accounts for mucin hyper-secretion in asthmatics subjects [14]. In addition to increased mucus secretion, the distributional pattern of mucin gene expression in respiratory tissues also seems to be altered in these airways pathologies [14, 15]. Because mucus overproduction is commonly accompanied by goblet cells hyperplasia, it is important to identify which mucins are expressed in the goblet cells of the nasal mucosa (NM) epithelium. Recent studies have demonstrated that NM epithelial goblet cells expressed *MUC5AC* [16, 17] and *MUC2* [12] mucins and that mucous cells in glands expressed *MUC5B* [18].

NP constitute an inflammatory disease, characterized by oedematous masses in the nasal cavities and paranasal sinuses affecting 2–4% of general population, whose aetiology is still unknown. Although commonly presented as a unique kind of disease, NP may occur in a variety of different diseases such as bilateral nasal polyposis, in patients with or without asthma and in which eosinophil infiltration constitutes a histological hallmark [19], NP in CF patients where neutrophils are supposed to be the main infiltrating inflammatory cell [20], and unilateral antrochoanal (AC) polyps where inflammatory cell infiltration seems to be less important [21].

As little is known about the heterogeneity of mucin gene expression in the healthy NM and in the different types of NP, the knowledge of these expression patterns may contribute to an improvement in the diagnosis and therapy for mucus hyper-secretion and/or mucosal inflammation. Thus, the aim of the present study was to investigate and compare mucin gene expression patterns, at both epithelial and glandular levels, in different types of nasal tissues including healthy NM, bilateral NP, NP from CF patients (CF polyps), and unilateral AC polyps in order to identify potential mucin expression patterns for specific upper airways pathologies.

Materials and methods

Subjects

Human NM was obtained from subjects ($n = 12$) undergoing nasal corrective surgery for septal deviation. Only one of the subjects was being treated with intranasal corticosteroids at the time of surgery. Bilateral NP were obtained from 38 patients undergoing nasal polypectomy. Among them, 22 patients presented a history of asthma, and 50% of the asthmatic patients ($N = 11$) presented a clear history of aspirin sensitivity. At the time of surgery, 19 patients (50%) were receiving both intranasal corticosteroids (budesonide, 400 µg twice daily) and oral prednisone (20 mg/day). Thirteen patients (34%) were atopic to common aeroallergens (Table 1). In the study, polyps from patients with CF ($N = 10$) and AC polyps ($N = 11$) were also analysed.

Antibodies

Monoclonal antibodies M8 and LDQ10, recognizing *MUC1* and *MUC2* respectively, were used as undiluted hybridoma supernatant for M8 [22] and as ascites fluid diluted 1 : 250 for LDQ10 [23]. B12 MoAb (Dr Castro, Barcelona, Spain), recognizing a synthetic dextran molecule, was used as negative control at 1/2 dilution. Polyclonal anti-*MUC4* [24], anti-*MUC6* [25], and anti-*MUC8* [6] antibodies, recognizing *MUC4*, *MUC6*, and *MUC8* respectively, were purified by affinity chromatography on the synthetic peptides coupled to AH-sepharose 4B (Pharmacia, Uppsala, Sweden). Rabbit polyclonal serum LUM5.1 [7], LUM5B.2 [26], and LUM7.1 [27] recognizing non-TR regions of *MUC5AC*, *MUC5B*, and *MUC7* respectively, were also used. Pre-immune rabbit serum was used as negative control at 1 : 1000 dilutions. Except for *MUC8*, specificity of all the antibodies has been described previously. The specificity of anti-*MUC8* affinity-purified antibodies was determined by ELISA and by peptide inhibition assays by immunohistochemistry on bronchial sections as described previously [28].

Table 1. Epidemiological characteristics of subjects and patients

Tissue	N	Age (years)*	Gender (M/F)	Steroid	
				treatment (yes/no)	Atopy (yes/no)
NM	12	32.3 ± 6.5	9/3	1/11	1/10 (1) [†]
CF	10	14.5 ± 6.7	7/3	2/8	0/2 (8)
AC polyps	11	41.4 ± 20.2	8/3	2/9	4/4 (3)
NP	38	55.9 ± 13.6	24/14	19/19	13/25
No Asthma	16	50.6 ± 14.5	11/5	8/8	5/11
Asthma	22	58.8 ± 13.5	13/9	11/11	8/14
ASA tolerance	11	49.5 ± 16.3	5/6	5/6	4/7
ASA Intolerance	11	51.7 ± 13.2	8/3	6/5	4/7

*Mean ± standard deviation. [†]Values in brackets represent unknown data. AC, antrochoanal; CF, cystic fibrosis; M, male, F, female; NM, nasal mucosa; NP, nasal polyp.

Table 2. Mucin protein expression in nasal tissues detected by immunohistochemistry

	NM		NP		CF		AC polyps	
	Positivity*	Rate (%)	Positivity	Rate (%)	Positivity	Rate (%)	Positivity	Rate (%)
Epithelium								
MUC1	12/12	100	35/35	100	10/10	100	10/10	100
MUC2	10/10	100	31/35	88.6	8/8	100	4/9	44.4
MUC4	9/12	75.0	35/35	100	10/10	100	10/10	100
MUC5AC	11/11	100	35/36	97.2	10/10	100	10/10	100
MUC5B	5/12	41.7	28/36	77.8	8/8	100	8/10	80.0
MUC6	5/11	45.5	0/37	0.0	0/10	0.0	0/10	0.0
MUC7	0/12	0.0	0/37	0.0	0/10	0.0	0/10	0.0
MUC8	10/10	100	37/37	100	9/9	100	10/10	100
Glands								
MUC1	11/11	100	25/25	100	7/7	100	2/2	100
MUC2	1/9	11.1	0/25	0.0	1/5	20.0	0/2	0.0
MUC4	6/11	54.5	21/29	72.4	7/9	77.8	1/2	50.0
MUC5AC	3/9	33.3	15/22	68.2	5/7	71.4	1/3	33.3
MUC5B	10/10	100	27/27	100	8/8	100	3/3	100
MUC6	5/11	45.5	2/24	8.3	1/7	14.3	0/3	0.0
MUC7	6/11	54.5	0/26	0.0	1/10	10.0	1/3	33.3
MUC8	11/11	100	27/28	96.4	8/8	100	2/2	100

*Values are expressed as positive cases /total cases.

AC, antrochoanal; CF, cystic fibrosis; NM, nasal mucosa; NP, nasal polyp.

Immunohistochemistry

The indirect immunoperoxidase technique was performed on 3 µm sections of paraffin-embedded tissue sections. Samples were dewaxed, rehydrated, treated with 0.01 M sodium citrate buffer at 100 °C for 5 min, and fixed in cold acetone for 10 min. Endogenous peroxidase was blocked with 4% hydrogen peroxide in phosphate-buffered saline (PBS) containing 0.1% sodium azide for 10 min. Non-specific-binding sites were blocked with 5% skim milk in PBS. Primary antibodies were diluted in 1% PBS-bovine serum albumin and applied for 90 min. After washing with PBS, the slides were incubated for 60 min with peroxidase-labelled secondary antibodies (DAKO, Glostrup, Denmark). Peroxidase reaction was developed using the diaminobenzidine Substrate-cromogen System (DAKO) according to the manufacturer's recommendations. The slides were counterstained with haematoxylin, dehydrated, and mounted with DPX (BDH, Poole, UK).

Sections were examined by light microscopy and the antibody staining patterns were scored in a quantitative manner. The pattern of reaction was classified in the epithelium and glands, and the number of positive cells was expressed as a percentage of the total number of cells (400 cells counted). The percentage of positive among total cases was also quantified (Table 2). A positive case was considered when more than 5% of positive cells were detected. The scoring of reactivity was independently determined by two observers (A.M. and C.d.B) in a blind manner.

In Situ Hybridization

Sense and antisense synthetic oligonucleotides (48 bp) corresponding to the tandem repeat sequences of mucin genes were labelled with digoxigenin (DIG) following the manufacturer's instructions (Boehringer, Mannheim, Germany). After surgery, tissue samples were immediately fixed in 4% paraformaldehyde (PFA), embedded in paraffin, and stored at 4 °C until use. Sections were deparaffinized and rehydrated. Proteinase K digestion (1 µg/mL in 0.1 M Tris HCl, pH 7, 0.5 M EDTA) was performed for 15 min at 37 °C. Samples were fixed with 4% PFA for 15 min and treated with 0.1 M triethanolamine, pH 8, and 0.25% acetic anhydride for 10 min. After pre-hybridization in 4 × SSPE (300 mM NaCl, 20 mM NaH₂PO₄, 2 mM EDTA) with 1% Denhardt's at 42 °C for 60 min, sections were dehydrated and hybridized overnight at 42 °C with DIG-labelled oligonucleotides (400 µg/mL) diluted in 4 SSPE, 1% Denhardt's, 50% formamide, 20 mM DTT, 0.1 M phosphate buffer, pH 7.2%, 1% sarkosyl, and tRNA (250 µg/mL). Post-hybridization washes were performed stepwise from 4 × SSPE at room temperature (RT) to a final wash with 0.1 × SSPE at 42 °C. Sections were dehydrated, dried and incubated with 5% normal horse serum for 3 h at RT. Alkaline phosphatase-conjugated sheep anti-DIG antibodies (Boehringer) were incubated overnight at 4 °C. Alkaline phosphatase was developed overnight at RT using nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (Promega, Madison, WI, USA) in 100 mM NaCl, 50 mM MgCl₂, 100 mM Tris HCl, pH 9.5. Slides were rinsed and mounted in Aquatex.

Table 3. Inflammatory component from healthy and pathologic tissues

Tissue	Lymphocyte (%)	Plasma cells (%)	Polymorphonuclear (%)	Eosinophils (%)
NM	40 (32.5–40)	50 (47.5–55)	5 (4–5)	5 (3.5–12.5)
NP	35 (15–45)	30 (25–40)*	5 (5–10)	25 (10–50)*
CFP	30 (20–36.3)	50 (48.8–61.3) [†]	5 (5–6.3)	10 (7.3–11.3) [†]
AC polyps	45 (21.3–65)	45 (22.5–50)	5 (3–8.8)	10 (2.5–18.8) [†]

* $P < 0.05$ compared with NM. [†] $P < 0.05$ compared with NP by the Mann–Whitney U -test.

Results are expressed as median and 25–75th percentile. NM, nasal mucosa; NP, nasal polyp; CFP, polyps from cystic fibrosis patients; AC, antrochoanal.

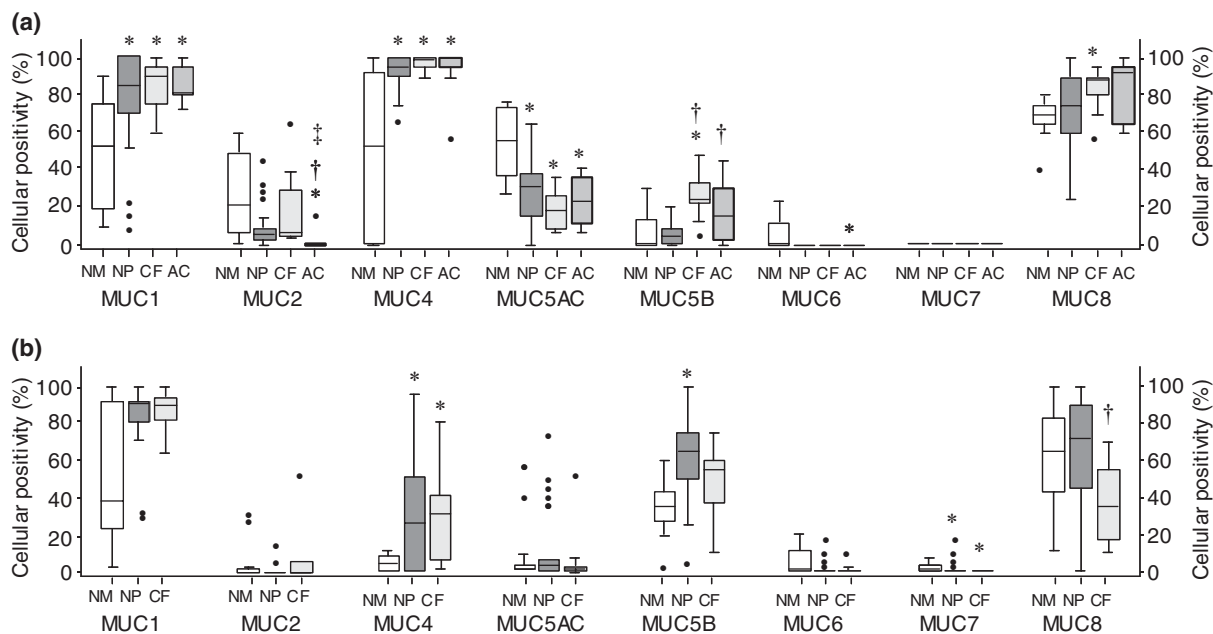


Fig. 1. Pattern of mucin gene expression in different types of upper airway tissues: nasal mucosa (NM), bilateral nasal polyps (NP), NP from patients having cystic fibrosis (CF), and antrochoanal (AC) polyps. The data are expressed as percentage of positive cells among total cells in the epithelium (a) and in the glands (b). Because of the absence of glands AC polyps are not represented in graph B. Box plots show the 25th, 50th (median), and 75th percentile values. Whiskers show the minimum and maximum values. Black points (●) represent outlier values. Comparisons were made using the Mann–Whitney U -test (* $P < 0.05$ vs. NM; [†] $P < 0.05$ vs. NP; [‡] $P < 0.05$ vs. CF).

The antisense oligonucleotide sequences used were as follows: MUC2 (5'-GGT CTG TGT GCC GGT GGG TGT TGG GGT TGG GGT CAC CGT GGT GGT GGT-3'), MUC4 (5'-GTC GGT GAC AGG AAG AGG GGT GGC GTG ACC TGT GGA TGC TGA GGA AGT-3'), MUC5AC (5'-AGG GGC AGA AGT TGT GCT CGT TGT GGG AGC AGA GGT TGT GCT GGT TGT-3'), and MUC6 (5'-CAT CTG TGC GTG GGT AGG GGT GAT GAC TGT GTG AGT ACT TGG AGT CAC-3'). The following tissues were used as controls: healthy colon for MUC2 and MUC4 and healthy stomach for MUC5AC and MUC6 [24, 28].

Inflammation

Inflammatory cells including eosinophils, lymphocytes, plasma cells, and polymorphonuclear cells were quantified, and the results were related to 100 inflammatory cells (Table 3).

Statistical Analysis

Mucin data were expressed as median and 25–75th percentile of positive cells among total cells. As the Kolmogorov–Smirnov test showed that most of the data did not reach a normal distribution, the non-parametric statistical Mann–Whitney's U -test was used for between-group comparisons. Rho Spearman's analysis was used to assess the correlation between mucin gene expression and inflammatory parameters in the different tissues. Statistical significance was set at $P < 0.05$.

Results

Mucin expression in human nasal mucosa

Epithelium. MUC1, MUC2, MUC5AC, and MUC8 mucins were detected in the epithelium of all cases (Table 2). The expression of MUC8 (median: 70%) was high, MUC1

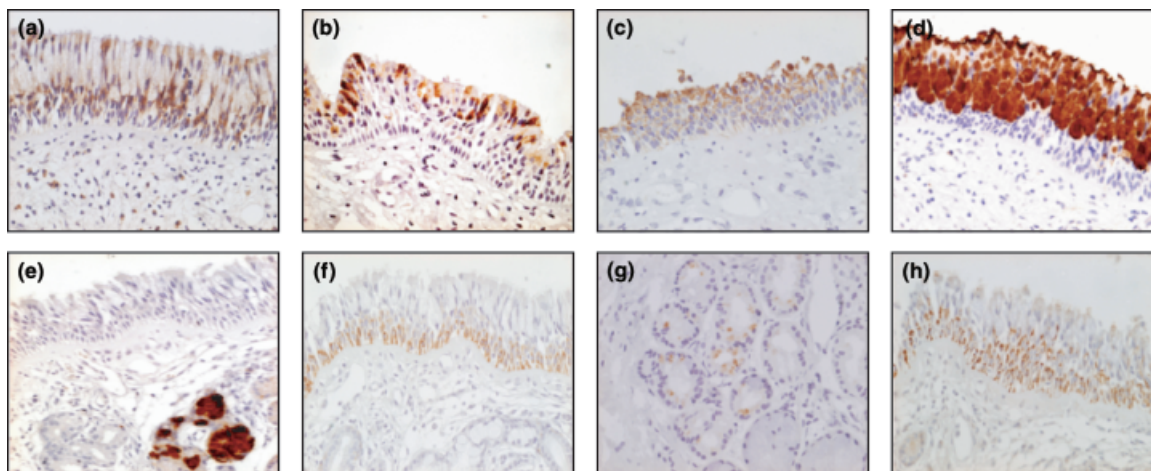


Fig. 2. (a)MUC1, (b) MUC2, (c) MUC4, (d) MUC5AC, (e) MUC5B, (f) MUC6, (g) MUC7, and (h) MUC8 mucins detected in human nasal mucosa by immunohistochemistry. MUC1, MUC4, and MUC8 were detected in nasal epithelial goblet and ciliated cells. MUC2 and MUC5AC were mainly detected in goblet cells. MUC5B was mainly detected in submucosal glands. MUC6 was detected at a low level while MUC7 was not expressed. (Original magnification: $\times 400$).

(52.5%) and MUC5AC (55%) expression was moderate, and MUC2 (21.3%) showed a low expression. MUC5B and MUC6 were detected in approximately half of the cases and the cellular positivity was similar for both mucins (Fig. 1A). MUC4 (52.5%) was variably detected in 75% of the samples while MUC7 was undetectable in the epithelium.

Submucosal glands. MUC1, MUC5B, and MUC8 were detected in all analysed cases (Table 2). MUC8 mucin was highly expressed (65%), MUC1 (40%), and MUC5B (37.5%) were moderately detected, and the rest of the mucins were lowly expressed, MUC2 being the mucin detected at the lowest level (Fig. 1b). The number of positive cells and positive cases for MUC5B and MUC7 was increased compared with the epithelium (Table 2).

According to the stained cellular type, MUC1 and MUC4 immunoreactivity was found in both mucus-secreting and ciliated cells, whereas MUC2 and MUC5AC staining was restricted to the mucus-secreting cells. MUC6 and MUC8 staining was limited to the basal area of the superficial epithelium, while MUC5B was mainly detected in mucus-secreting cells of submucosal glands. MUC7 was undetectable in the epithelium although it was focally detected in glands (Fig. 2).

Mucin expression in nasal polyps

Epithelium. MUC1, MUC4, and MUC8 mucins were highly detected in all studied samples. MUC5AC was moderately expressed while MUC2 and MUC5B were poorly expressed, and MUC6 and MUC7 were not detected (Fig. 1a; Table 2).

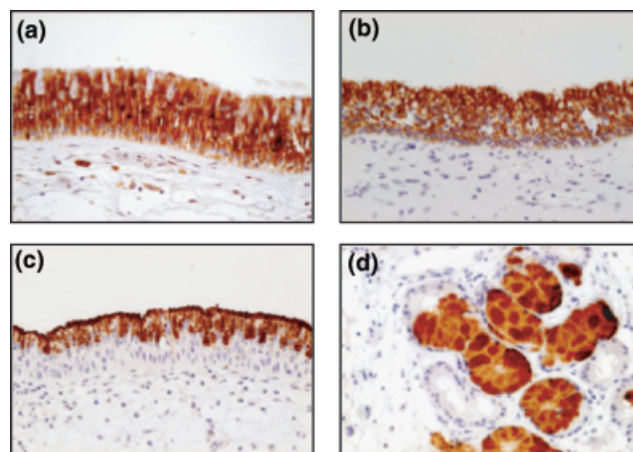


Fig. 3. Immunohistochemistry of mucin expression in bilateral nasal polyps: (a) MUC1, (b) MUC4, (c) MUC5AC, and (d) MUC5B. MUC1 and MUC4 were highly expressed throughout the epithelium. MUC5AC and MUC5B are mainly expressed in epithelial and glandular mucous cells, respectively. (Original magnification: $\times 400$)

Submucosal glands. MUC1 and MUC8 were also highly expressed in all the cases. The expression of MUC5AC was decreased whereas MUC5B was increased compared with the epithelium (Fig. 1b). In contrast to a high positivity in the epithelium (90%), MUC2 was not detected in glands. While MUC6 was detected in few samples, MUC7 was totally absent (Table 2).

Expression pattern. Comparing the mucin expression pattern in NP with that found in healthy NM, an increased expression of MUC4 in both the polyp epithelium (95%; $P < 0.001$) and glands (25%; $P < 0.05$) as well as an increase of MUC1 (85%; $P < 0.001$) in the epithelium was observed. The expression of MUC5AC (30%; $P < 0.001$)

and MUC6 (0%; $P < 0.001$) was decreased in NP epithelium, whereas MUC7 (0%; $P < 0.01$) was decreased in NP glands. MUC5B (65%; $P < 0.001$) mucin was increased in NP glands with respect to NM (Fig. 3).

When comparing the mucin expression between asthma/no-asthma patients, a lower MUC2 mucin expression in asthmatics (3.8%; $P < 0.01$) than in non-asthmatic (10%) subjects was found in nasal epithelium. The expression of MUC5B was higher in NP glands from asthmatics patients with aspirin tolerance (67.5%; $P < 0.01$) than in polyps of patients with aspirin sensitivity (50%). Polyps of patients receiving corticosteroid treatment showed a higher expression of MUC1 (95%; $P < 0.05$) and MUC5B (7.5%; $P = 0.01$) but a lower expression of MUC8 (65%; $P = 0.01$) than the non-treated group (80%, 1.3%, and 87.5%, respectively) (Fig. 3). No differences in mucin expression between atopic and non-atopic subjects were found (data not shown).

Mucin expression in nasal polyps from cystic fibrosis patients

Epithelium. All the studied mucins, except MUC6 and MUC7, were detected in 100% of the cases (Table 3). The expression of mucins was high for MUC1, MUC4, and MUC8, moderate for MUC5B, and low for MUC2 and MUC5AC, while MUC6 and MUC7 were not expressed (Figs 1a and 4).

Submucosal glands. The number of positive cases was markedly decreased for MUC2 mucin compared with the epithelium (Table 3). The expression was high for MUC1, moderate for MUC4, MUC5B, and MUC8, and very low for MUC2, MUC5AC, and MUC7.

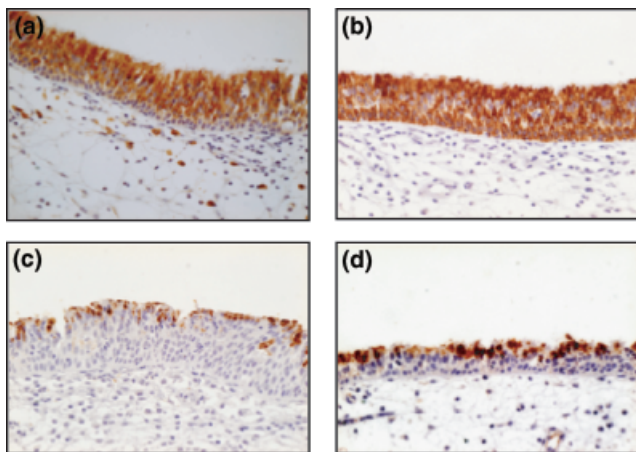


Fig. 4. Immunohistochemistry of mucin expression in nasal polyps from cystic fibrosis patients: (a) MUC1, (b) MUC4, (c) MUC5AC, and (d) MUC5B. MUC1 and MUC4 were highly expressed throughout the epithelium, whereas MUC5AC and MUC5B were mainly expressed in goblet cells. (Original magnification: $\times 400$).

Expression pattern. When the mucin expression pattern detected in NP from CF patients, was compared with that found in healthy NM, an increased expression of MUC1 (90%; $P < 0.001$), MUC5B (25%; $P < 0.01$), and MUC8 (90%; $P < 0.001$) was observed in the epithelium. MUC4 expression was increased in both the epithelium (100%; $P < 0.001$) and the glands (30%; $P < 0.05$). MUC5AC expression was significantly lower (17.5%; $P < 0.001$) than in NM (Fig. 1). When comparing the mucin expression between CF polyps and bilateral NP, an increased expression of MUC5B mucin was observed in CF polyps epithelium ($P < 0.001$) and decreased MUC8 in glands ($P < 0.05$).

Mucin expression in antrochoanal polyps

Epithelium. MUC1, MUC4, and MUC8 mucins were detected in all the analysed cases in a high proportion of cells (Table 3). MUC5AC was also detected in all the samples but the expression intensity was low. The expression of MUC5B and MUC2 was low, while MUC6 and MUC7 were not detected (Fig. 1A).

Submucosal glands. Because of the low number of glands found in AC polyp samples, the cellular positivity could not be assessed at the glandular level.

Expression pattern. When the pattern of mucin expression in AC polyps was compared with that of healthy NM, an increased expression of MUC1 (80%; $P < 0.01$) and MUC4 (100%; $P = 0.001$) as well as a decreased expression of MUC2 (0%, 0–1.3; $P < 0.001$) and MUC5AC (22.5%; $P = 0.001$) was observed. MUC2 mucin expression was also decreased compared with CF polyps and bilateral NP (both

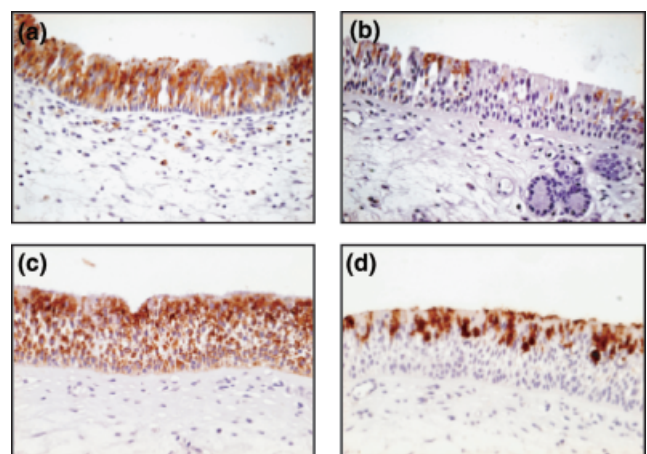


Fig. 5. Immunohistochemistry of mucin expression in AC polyps: (a) MUC1, (b) MUC2, (c) MUC4, and (d) MUC5AC. MUC1 and MUC4 were highly detected throughout the epithelium, whereas MUC2 and MUC5AC were mainly detected in goblet cells. (Original magnification: $\times 400$).

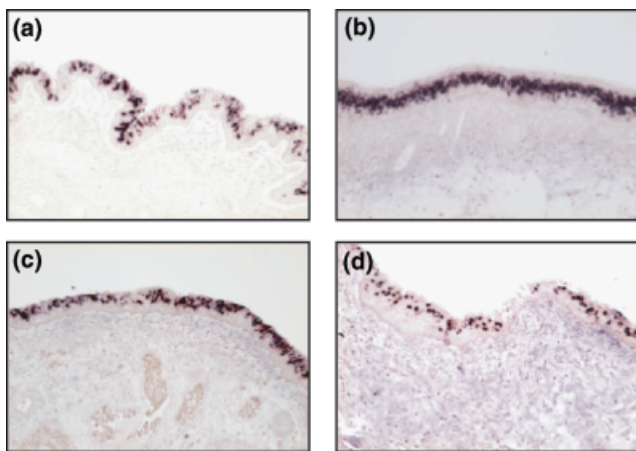


Fig. 6. MUC2, MUC4, and MUC5AC transcripts were detected by *in situ* hybridization. (a and c) MUC2 and MUC5AC transcripts expressed in nasal mucosa. (b and d) MUC4 and MUC5AC transcripts expressed in a bilateral nasal polyp. (Original magnification: $\times 150$).

$P < 0.001$), while MUC5B was increased in AC polyps compared with bilateral NP ($P < 0.05$) (Figs 1a and 5).

Detection of mucin transcripts by *in situ* hybridization

To confirm the immunohistochemical findings at the RNA level, *in situ* hybridization for MUC2, MUC4, MUC5AC, and MUC6 was carried out on selected samples of healthy NM ($n = 4$) and bilateral NP ($n = 5$). MUC2 mRNA was expressed in the epithelium of NM in a low proportion of cells (Fig. 6A), but not in bilateral NP. MUC4 was expressed in all samples in a high proportion of epithelial cells (Fig. 6B). MUC5AC was mainly detected in goblet cells in both NM and polyps, although the expression was higher in NM than in NP (Figs 6C and D). MUC6 was not detected in either NM or polyps (data not shown).

Inflammation

The analysis of the haematoxylin–eosin staining showed a higher percentage of eosinophils in bilateral NP (25%; $P < 0.05$) than in the other tissues (NM = 5%; CF and AC polyps = 10%) (Table 3). Bilateral NP from patients receiving corticoids treatment (10%) showed a decreased eosinophil infiltration compared with NP from non-treated patients (50%; $P < 0.001$) (data not shown). There were no significant differences in the polymorphonuclear cells and lymphocyte content in the different tissues (Table 3). As a high variability in the correlations between mucin expression and inflammatory cells were found, no correlation data was reported.

Discussion

In the present study, the pattern of mucin gene expression in healthy NM has been analysed and compared with that

of bilateral NP, NP from CF patients and AC polyps in order to assess the potential role of the mucins as pathologic markers for diagnosis and disease evolution in patients with chronic rhinosinusitis and NP. Our results indicate that an altered expression pattern is present in all NP samples.

Mucins in healthy nasal mucosa

In healthy NM, the major mucins expressed are MUC1, MUC4, MUC5AC, and MUC8 in the epithelium, and MUC1, MUC5B, and MUC8 in the glands. Previous data have reported that MUC4 and MUC5AC are the most highly expressed mucins in healthy inferior turbinates [5] and that MUC5AC and MUC5B are the two major mucin populations in respiratory secretions [7, 8]. MUC5AC has been exclusively detected in epithelial goblet cells of NM, while MUC5B has also been found in the mucous cells of submucosal glands [16]. In addition, MUC5AC has been also localized in goblet cells from other respiratory epithelia such as the bronchi, trachea, and lungs [6, 29, 30], as well as in non-respiratory tissues such as stomach [25] and gallbladder epithelia [31]. Little data regarding a mucin expression in the upper airways have been published. MUC2 and MUC7 were detected at a low level, while MUC6 was absent [5]. These observations are in accordance with our findings, in which MUC6 and MUC7 were exclusively detected in healthy NM at very low levels, and MUC2 was scarcely expressed in both healthy and pathological nasal tissues.

Mucins in nasal polyps

Several authors have analysed the expression of mucin genes in different samples of upper airway mucosa. For instance, an increase of MUC8 mRNA and a decrease of MUC5AC mRNA expression in bilateral NP [13], as well as an up-regulation of MUC8 in chronic rhinosinusitis mucosa have been found, at both mRNA and protein levels [32]. By contrast, several studies have described an increased expression of MUC5AC in bilateral NP that can be associated to goblet cells hyperplasia [17, 33]. In this way, if hyperplastic epithelium appeared in pathologic tissues, the low percentage of MUC5AC-positive cells detected in our samples could be explained by either a higher proliferation of non-goblet cells (ciliated and basal) with respect to goblet cells or by the presence of less mature goblet cells with a differential phenotype. On the other hand, Kim et al. [18] have recently demonstrated an up-regulation of MUC5B mRNA expression in chronic rhinosinusitis mucosa compared with normal sinus mucosa. This result is comparable with our finding of increased MUC5B expression in bilateral NP glands.

Mucins in nasal polyp from various origins

In CF polyps, an up-regulation of MUC5AC mRNA in both human NP and bronchial tissues of CF subjects has been

reported [15], as well as an increase of MUC2 mRNA expression in CF NM [34]. However, we detected a decreased expression of MUC5AC and MUC2 in NP of CF patients similar to that present in bilateral NP. A potential explanation for the different results may be that all CF patients in the study of Li et al. were included in a gene therapy trial receiving either CFTR cDNA-liposome complexes or liposome alone by nasal topical application. The administration of liposomes may affect the surface of the epithelium and up-regulated MUC2 expression, as it has already been described for other inflammatory mediators [35]. On the other hand, our findings agree with those of Voynow and Henke, who detected a decreased expression of MUC5AC in nasal epithelial cells and sputum from CF subjects compared with NHNE cells and sputum of healthy subjects [36, 37].

In addition, our study reports, for the first time, the mucin gene expression in AC polyps. The results indicate that in this pathologic tissue, the mucin expression pattern is similar to that found in bilateral and CF NP, except for MUC2, which was found to be down-regulated in AC polyps.

Mucins in respiratory inflammation

Although several studies have tried to elucidate the role of inflammatory cells and their mediators in the regulation of mucin genes, the relationship between inflammation and mucin overproduction and/or hyper-secretion is still poorly understood. It has been reported that inflammatory cells present in NP could play an important role in the disease [38]. One of these implications could be the regulation of the mucin genes as it has been described in other respiratory cellular models. For example, Burgel et al. demonstrated that eosinophil products increased mucin production in airway epithelial cells [39] and pro-inflammatory cytokines (IL-1 β , IL-9, and TNF- α) can regulate the expression of specific mucin genes (MUC2, MUC5AC, and MUC8) in bronchial epithelia cells, regulation that is mediated through a mechanism involving the ERK/MAPK/RSK1/CREB pathway [40–42]. In our tissue samples, bilateral and CF NP showed a higher inflammatory component compared with healthy NM and AC NP and eosinophils were the major inflammatory cells detected in bilateral NP. Altogether, these data could indicate that MUC1, MUC4, and MUC5B up-regulation, observed in bilateral NP and to a lower extent in CF and in AC NP, might be due to the increase of eosinophil infiltration, wherein activation and secretion of inflammatory factors would regulate the expression of specific mucin genes. However, factors secreted by other inflammatory cells present in NP, such as neutrophils, could also contribute to regulation of mucin gene expression [33].

The increased expression of secreted mucins may contribute to mucus hyper-secretion and to mucus viscoelas-

ticity changes. On the other hand, the biologic significance of the membrane mucin (MUC1 and MUC4) activation in pathologic tissues may be related to the implication of these mucins for intracellular signalling pathways related to proliferative processes. MUC4 may carry out this action through the epidermal growth factor-like domains present in its sequence, which interact with ErbB2 [43]. Therefore, membrane mucins could be involved in epithelial cell hyperplasia reported to occur in several respiratory pathologies.

Recently, it has been shown that intranasal steroids decrease the number of eosinophils but not the level of mucins expression in bilateral NP [44]. Although the effect of corticosteroids on mucin gene expression was not the main aim of the present study, we found some remarkable differences between bilateral NP from steroid-treated and non-treated patients. In the treated group, a decrease in the number of eosinophils was detected in association with a decrease of MUC8 expression, suggesting that eosinophils could be involved in the regulation of MUC8.

Conclusions

These results suggest that bilateral NP have a different pattern of mucin expression, an increase of MUC1 and MUC4 and a decrease of MUC5AC, than healthy NM. Among NP, CF polyps, with an increase in MUC5B, and AC polyps, with a decrease in MUC2, also show a differential mucin expression pattern than bilateral nasal polyposis. These findings, together with further studies on the regulation of mucins by pro-inflammatory agents and anti-inflammatory drugs, might aid in the differential diagnosis and improved therapies of respiratory diseases with NP.

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References

- 1 Rose MC, Lynn WS, Kaufman B. Resolution of the major components of human lung mucosal gel and their capabilities for reaggregation and gel formation. *Biochemistry* 1979; 18: 4030–7.
- 2 Rose MC, Nickola TJ, Voynow JA. Airway mucus obstruction: mucin glycoproteins, MUC gene regulation and goblet cell hyperplasia. *Am J Respir Cell Mol Biol* 2001; 25:533–7.
- 3 Gum JR Jr, Crawley SC, Hicks JW, Szymkowski DE, Kim YS. MUC17, a novel membrane-tethered mucin. *Biochem Biophys Res Commun* 2002; 291:466–75.

- 4 Pallesen LT, Berglund L, Rasmussen LK, Petersen TE, Rasmussen JT. Isolation and characterization of MUC15, a novel cell membrane-associated mucin. *Eur J Biochem* 2002; **269**: 2755–63.
- 5 Aust MR, Madsen CS, Jennings A, Kasperbauer JL, Gendler SJ. Mucin mRNA expression in normal and vasomotor inferior turbinates. *Am J Rhinol* 1997; **11**:293–302.
- 6 Lopez-Ferrer A, Curull V, Barranco C *et al*. Mucins as differentiation markers in bronchial epithelium. Squamous cell carcinoma and adenocarcinoma display similar expression patterns. *Am J Respir Cell Mol Biol* 2001; **24**:22–9.
- 7 Hovenberg JW, Davies JR, Carlstedt I. Different mucins are produced by the surface epithelium and the submucosa in human trachea: identification of MUC5AC as a major mucin from the goblet cells. *Biochem J* 1996; **318**:319–24.
- 8 Wickstrom C, Davies JR, Eriksen GV, Veerman EC, Carlstedt I. MUC5B is a major gel-forming, oligomeric mucin from human salivary gland, respiratory tract and endocervix: identification of glycoforms and C-terminal cleavage. *Biochem J* 1998; **334**: 685–93.
- 9 Pigny P, Guyonnet-Duperat V, Hill AS *et al*. Human mucin genes assigned to 11p15.5: identification and organization of a cluster of genes. *Genomics* 1996; **38**:340–52.
- 10 Gum JR Jr. Human mucin glycoproteins: varied structures predict diverse properties and specific functions. *Biochem Soc Trans* 1995; **23**:795–9.
- 11 Shimura S, Andoh Y, Haraguchi M, Shirato K. Continuity of airway goblet cells and intraluminal mucus in the airways of patients with bronchial asthma. *Eur Respir J* 1996; **9**: 1395–401.
- 12 Kim SS, Kim KS, Lee JG, Park IY, Koo JS, Yoon JH. Levels of intracellular protein and messenger RNA of mucin and lysozyme in normal human nasal and polyp epithelium. *Laryngoscope* 2000; **110**:276–80.
- 13 Seong JK, Koo JS, Lee WJ *et al*. Upregulation of MUC8 and downregulation of MUC5AC by inflammatory mediators in human nasal polyps and cultured nasal epithelium. *Acta Otolaryngol* 2002; **122**:401–7.
- 14 Ordoñez CL, Khashayar R, Wong HH *et al*. Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am J Respir Crit Care Med* 2001; **163**:517–23.
- 15 Dohrman A, Miyata S, Gallup M *et al*. Mucin gene (MUC 2 and MUC 5AC) upregulation by Gram-positive and Gram-negative bacteria. *Biochim Biophys Acta* 1998; **1406**:251–9.
- 16 Gronenberg DA, Peiser C, Dinh QT *et al*. Distribution of respiratory mucin proteins in human nasal mucosa. *Laryngoscope* 2003; **113**:520–4.
- 17 Kim CH, Song KS, Kim SS, Kim HU, Seong JK, Yoon JH. Expression of MUC5AC mRNA in the Goblet Cells of Human Nasal Mucosa. *Laryngoscope* 2000; **110**:2110–3.
- 18 Kim DH, Chu HS, Lee JY, Hwang SJ, Lee SH, Lee HM. Up-regulation of MUC5AC and MUC5B mucin genes in chronic rhinosinusitis. *Arch Otolaryngol Head Neck Surg* 2004; **130**:747–52.
- 19 Lamblin C, Gosset P, Salez F *et al*. Eosinophilic airway inflammation in nasal polyposis. *J Allergy Clin Immunol* 1999; **104**:85–92.
- 20 Rowe-Jones JM, Shembekar M, Trendell-Smith N, Mackay IS. Polypoidal rhinosinusitis in cystic fibrosis: a clinical and histopathological study. *Clin Otolaryngol* 1997; **22**:167–71.
- 21 Maldonado M, Martinez A, Alobid I, Mullol J. The antrochoanal polyp. *Rhinology* 2004; **42**:178–82.
- 22 Price MR, Edwards S, Owainati A *et al*. Multiple epitopes on a human breast-carcinoma-associated antigen. *Int J Cancer* 1985; **36**:567–74.
- 23 Gambus G, de Bolos C, Andreu D. Detection of the MUC2 apomucin tandem repeat with a mouse monoclonal antibody. *Gastroenterology* 1993; **104**:93–102.
- 24 Lopez-Ferrer A, de Bolos C, Barranco C *et al*. Role of fucosyltransferases in the association between apomucin and Lewis antigen expression in normal and malignant gastric epithelium. *Gut* 2000; **47**:349–56.
- 25 De Bolos C, Garrido M, Real FX. MUC6 apomucin shows a distinct normal tissue distribution that correlates with Lewis antigen expression in the human stomach. *Gastroenterology* 1995; **109**:723–34.
- 26 Wickstrom C, Carlstedt I. N-terminal cleavage of the salivary MUC5B mucin. Analogy with the Van Willebrand propeptide? *J Biol Chem* 2001; **276**:47116–21.
- 27 Wickstrom C, Christersson C, Davies JR, Carlstedt I. Macromolecular organization of saliva: identification of 'insoluble' MUC5B assemblies and non-mucin proteins in the gel phase. *Biochem J* 2000; **351**:421–8.
- 28 De Bolos C, Guma M, Barranco C, Garrido M, Kim YS, Real FX. MUC6 expression in breast tissues and cultured cells: abnormal expression in tumors and regulation by steroid hormones. *Int J Cancer* 1998; **77**:193–9.
- 29 Groneberg DA, Eynott PR, Lim S *et al*. Expression of respiratory mucins in fatal status asthmaticus and mild asthma. *Histopathology* 2002; **40**:367–73.
- 30 Vinall LE, Fowler JC, Jones AL *et al*. Polymorphism of human mucin genes in chest disease: possible significance of MUC2. *Am J Respir Cell Mol Biol* 2000; **23**:678–86.
- 31 Sasaki M, Yamato T, Nakanuma Y, Ho SB, Kim YS. Expression of MUC2, MUC5AC and MUC6 apomucins in carcinoma, dysplasia and non-dysplastic epithelia of the gallbladder. *Pathol Int* 1999; **49**:38–44.
- 32 Lee HM, Kim DH, Kim JM, Lee SH, Hwang SJ. MUC8 mucin gene up-regulation in chronic rhinosinusitis. *Ann Otol Rhinol Laryngol* 2004; **113**:662–6.
- 33 Burgel PR, Escudier E, Coste A *et al*. Relation of epidermal growth factor receptor expression to goblet cell hyperplasia in nasal polyps. *J Allergy Clin Immunol* 2000; **106**: 705–12.
- 34 Li D, Wang D, Majumdar S *et al*. Localization and up-regulation of mucin (MUC2) gene expression in human nasal biopsies of patients with cystic fibrosis. *J Pathol* 1997; **181**: 305–10.
- 35 Li JD, Dohrman AF, Gallup M *et al*. Transcriptional activation of mucin by *Pseudomonas aeruginosa* lipopolysaccharide in the pathogenesis of cystic fibrosis lung disease. *Proc Natl Acad Sci USA* 1997; **94**:967–72.
- 36 Henke MO, Renner A, Huber RM, Seeds MC, Rubin BK. MUC5AC and MUC5B Mucins Are Decreased in Cystic Fibrosis Airway Secretions. *Am J Respir Cell Mol Biol* 2004; **31**:86–91.
- 37 Voynow JA, Selby DM, Rose MC. Mucin gene expression (MUC1, MUC2, and MUC5/5AC) in nasal epithelial cells of cystic fibrosis, allergic rhinitis, and normal individuals. *Lung* 1998; **176**: 345–54.

- 38 Hamilos DL, Leung DY, Wood R *et al.* Eosinophil infiltration in nonallergic chronic hyperplastic sinusitis with nasal polyposis (CHS/NP) is associated with endothelial VCAM-1 upregulation and expression of TNF-alpha. *Am J Respir Cell Mol Biol* 1996; 15:443-50.
- 39 Burgel PR, Lazarus SC, Tam DC *et al.* Human eosinophils induce mucin production in airway epithelial cells via epidermal growth factor receptor activation. *J Immunol* 2001; 167:5948-54.
- 40 Louahed J, Toda M, Jen J *et al.* Interleukin-9 upregulates mucus expression in the airways. *Am J Respir Cell Mol Biol* 2000; 22:649-56.
- 41 Song KS, Seong JK, Chung KC *et al.* Induction of MUC8 gene expression by interleukin-1 beta is mediated by a sequential ERK MAPK/RSK1/CREB cascade pathway in human airway epithelial cells. *J Biol Chem* 2003a; 278:34890-6.
- 42 Song KS, Lee WJ, Chung KC *et al.* Interleukin-1 beta and tumor necrosis factor-alpha induce MUC5AC overexpression through a mechanism involving ERK/p38 mitogen-activated protein kinases-MSK1-CREB activation in human airway epithelial cells. *J Biol Chem* 2003b; 278:23243-50.
- 43 Carraway KL, Rossi EA, Komatsu M *et al.* An intramembrane modulator of the ErbB2 receptor tyrosine kinase that potentiates neuregulin signaling. *J Biol Chem* 1999; 274:5263-6.
- 44 Burgel PR, Cardell LO, Ueki IF, Nadel JA. Intranasal steroids decrease eosinophils but not mucin expression in nasal polyps. *Eur Respir J* 2004; 24:594-600.