

ORIGINAL ARTICLE

Rhinitis, Sinusitis, and Upper Airway Disease

Th2 cytokines orchestrate the secretion of MUC5AC and MUC5B in IL-5-positive chronic rhinosinusitis with nasal polyps

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Abstract

Background: Mucin over-secretion is a significant characteristic of chronic rhinosinusitis with nasal polyps (CRSwNP). This study aimed to investigate the relationship between Th2 cytokines and MUC5AC or MUC5B, and the mechanism of mucin over-secretion in the type-2 inflammatory endotype of CRSwNP.

Methods: Main Th-cell cytokines, associated mediators, and mucins were determined in the homogenates of nasal polyp samples from 21 CRSwNP patients and inferior turbinate samples from 8 controls, by ELISA or UniCAP system. Secretion of MUC5AC and MUC5B was measured in the supernatants of IL-5, IL-4, or IL-13 primed nasal polyp fragments. Co-localization of MUC5AC, MUC5B, and IL-4 receptor α (IL-4R α) in CRSwNP and controls was evaluated by immunohistochemistry. Gene expression of IL-4R α in the samples was measured by real-time reverse transcription-polymerase chain reaction.

Results: Baseline protein levels of the Th2-cytokines IL-4, IL-5, and IL-13, and mucins MUC5AC and MUC5B were significantly higher in the IL-5(+) CRSwNP group, compared to control and IL-5(-) CRSwNP groups. MUC5AC and MUC5B secretions were significantly increased in IL-4- or IL-13-primed, but not IL-5-primed fragments of nasal polyps. Immuno-stained serial sections demonstrated that IL-4R α was widely expressed in the epithelium and submucosal glands in control and nasal polyp tissues. Gene expression of IL-4R α was elevated in nasal polyp tissues, specifically in the IL-5(+) CRSwNP group.

Conclusions: In type-2 inflammatory nasal polyps, characterized by the tissue expression of IL-5, MUC5AC and MUC5B are overexpressed. Both IL-4 and IL-13 may upregulate mucin expression via IL-4R α , which is also overexpressed in IL-5(+) CRSwNP.

KEYWORDS

chronic rhinosinusitis, cytokines, MUC5AC, MUC5B, nasal polyp

1 | INTRODUCTION

Airway mucus plays an important role in the protection against airborne irritants, particles, and microorganisms.¹ However, the persistent accumulation of mucus resulting from overproduction or decreased clearance can lead to infection and inflammation by providing an environment for microbial growth.² The quality and quantity of mucins present in the mucus determine the viscoelastic property of mucus, which plays major role in normal mucociliary function.³ Chronic rhinosinusitis with nasal polyp (CRSwNP) is thought to be associated with mucus hypersecretion, followed by rhinorrhea, as the main clinical symptom.⁴ Some researchers have investigated the pattern of mucin gene expression in healthy nasal tissue and nasal polyps from patients with rhinitis and rhinosinusitis, and found that MUC1, MUC4, MUC5AC, and MUC5B were highly expressed in nasal polyp tissues compared to healthy nasal tissue.^{5–7} Furthermore, treatment for 12 weeks with intranasal corticosteroids could downregulate MUC5AC and MUC5B production in the epithelium and glands, respectively, in tissue of nasal polyposis patients.⁷ Although the disease is generally treated by surgery and systematic medical intervention, symptoms in some of patients are still not improved dramatically^{8,9} indicating that phenotypes of CRSwNP based on clinical criteria are not sufficient to reveal the underlying inflammatory mechanisms.^{10,11}

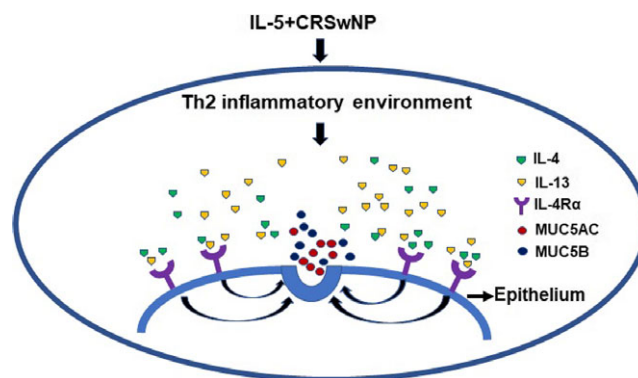
Endotyping of CRSwNP, based on molecular mechanisms, has received increasing attention in recent years. Different inflammatory patterns determined by Th cytokines and their downstream events, such as tissue eosinophilia or neutrophilia, define the classification of endotypes of CRSwNP.^{12,13} Th-cell cytokine patterns of patients with CRSwNP are quite different amongst the ethnic groups and locations. Zhang et al¹⁴ demonstrated that Chinese CRSwNP patients are characterized with a Th1/Th17 polarization and significantly increased levels of IL-17A and interferon γ . One recent study has demonstrated that CRSwNP patients from Benelux, Berlin, Adelaide and Tochigi were Th2 biased, whereas CRSwNP patients from Beijing mainly demonstrated Th2/Th1/Th17 mixed patterns.¹⁵

Although nasal polyps from CRSwNP patients have been shown to possess an abnormality of mucin over-secretion and the different endotypes are defined by Th cytokine biased inflammatory patterns, presently little is known about the relationship between mucin over-secretion and endotypes of nasal polyps from CRSwNP patients. The aim of our study was therefore to investigate the expression of MUC5AC and MUC5B in nasal polyp tissues of patients with different endotypes of CRSwNP and further analyze the putative mechanisms underlying MUC5AC and MUC5B over-secretion.

2 | MATERIALS AND METHODS

2.1 | Patients

Patients were recruited at the department of Otorhinolaryngology of the Ghent University Hospital, Belgium. All patients were Caucasian



GRAPHICAL ABSTRACT

The highlight of this study lies in the finding that Th2 inflammatory patterns are strongly related to mucin hypersecretion in endotypes of CRSwNP defined by IL-5. In the present study, we found that IL-4, IL-13, MUC5AC and MUC5B were overexpressed in IL-5(+) CRSwNP. Th2 associated mediators IgE and ECP were significantly increased in IL-5(+) CRSwNP as well and intimately correlated to mucin hypersecretion. Both IL-4 and IL-13 may upregulate mucin expression via their common receptor, IL-4R α , which is also overexpressed in IL-5(+) CRSwNP.

and aged between 18 and 66 years. Samples from 21 patients suffering from chronic rhinosinusitis (CRSwNP) were obtained during functional endoscopic sinus surgery procedures. The diagnosis of sinus disease was based on history, clinical examination, nasal endoscopy, and computed tomography of the paranasal cavities according to the current European EPOS guidelines.¹⁶ All patients underwent a skin prick test to common inhalant allergens and terminated oral and nasal corticoids at least 4 weeks before operation. Similarly, inferior turbinate samples from 8 patients without sinus disease undergoing septoplasty or rhinoseptoplasty were collected as controls. Demographic and clinical characteristics of the 2 patient groups are shown in Table 1.

The study was approved by the local Ethical committee of the University Hospital Ghent, Belgium. A written informed consent was obtained from each patient before inclusion in the study.

2.2 | Measurement of mediators in tissue homogenates

Measurements of mediators were performed on tissue homogenates as previously described.¹⁷ Supernatants collected after centrifugation of the homogenate samples were assayed for IL-4, IL-5, IL-13, IL-17, IFN- γ , myeloperoxidase (MPO), monocyte and chemotactic protein 1 (MCP-1), using commercially available ELISA kits (Quantikine ELISA, R&D Systems, UK; MPO was from Oxis International, USA; IL-4 and IL-13 were from Bio-rad Laboratories Inc., USA). Similarly, eosinophilic cationic protein (ECP) and total IgE were measured using the UniCAP system (Phadia, Uppsala, Sweden), and MUC5AC and MUC5B were determined using ELISA kits available from USCN Life Science, Wuhan, China.

TABLE 1 Patient characteristics

Demographic/Clinical feature	Control n = 8	CRSwNP n = 21	P value
Age (y), Mean $\pm$ SD (range)	31.3 $\pm$ 11.5 (18-57)	44.6 $\pm$ 8.9(21-66)*	.003
Female, n (%)	4 (50.0%)	8 (38.1%)	.683
Smoking, n (%)	3 (37.5%)	8 (38.1%)	1.000
Skin prick test positive, n (%)	2 (25.0%)	10 (47.6%)	.408
Sensitization profile			
Dermatophagoides pteronyssinus	2/2	8/10	
Dermatophagoides farinae	2/2	6/10	
Grass mix	1/2	5/10	
Cat	-	2/10	
Dog	-	4/10	
Blattella	-	3/10	
Artemisia	-	5/10	
Comorbidities	3	9	1.000
Hypertension	2/8	5/21	
Diabetes	1/8	3/21	
Heart disease	-	1/21	
Asthma, n (%)	0	6 (28.6%)	.148

A clinician-based diagnosis of asthma was made according to the criteria of the Global Initiative for Asthma.³³ P values signs after Fisher's exact test or Student's t test means as follow: *P < .01 in Control vs CRSwNP.

2.3 | Immunohistochemistry

Freshly obtained sinonasal mucosa tissues from control subjects and patients with CRSwNP were assessed for the presence of MUC5AC, MUC5AB, and IL-4R α by immunohistochemistry. Briefly, all samples were fixed with 10% paraformaldehyde (pH 7.4), embedded in paraffin, and cut into 4 μ m thick sections. After autoclave pretreatment, the sections were incubated with 3% hydrogen peroxide, rinsed in phosphate buffered solution (PBS), and incubated overnight at 4°C with MUC5AC antibody (mouse monoclonal; 1:100; Abcam, Cambridge, MA, USA), MUC5B antibody (mouse monoclonal; 1:100; Abcam, Cambridge, MA, USA), and Human IL-4R α antibody (mouse monoclonal; 1:100; R&D systems, Minneapolis, USA). The sections were subsequently incubated with peroxidase conjugated secondary antibody for 40 minutes at 37°C, and at the end of incubation, the peroxidase activity was visualized by a color reaction using diaminobenzidine (DAB) as the substrate. The slides were counterstained with hematoxylin and examined using an Olympus BX51 microscope (Tokyo, Japan).

2.4 | Ex-vivo human sinonasal tissue fragment stimulation

The effect of treatment with IL-4, IL-5, or IL-13 on secretion of MUC5AC and MUC5B by nasal polyps was investigated using the "human sinonasal tissue fragment stimulation" assay developed in-house, as described by Patou et al.¹⁸ Briefly, human nasal polyp tissue was cut into small pieces in RPMI 1640 tissue culture medium (Sigma Aldrich, Bornem, Belgium) containing 2 mmol/L/L-glutamine

(Invitrogen, Belgium), 50 IU/mL penicillin, 50 mg/mL streptomycin (Invitrogen), and 0.1% BSA (Sigma Aldrich), and subsequently passed through a mesh yielding fragments of equal size ($\pm$ 0.9 mm³). The tissue fragments were allowed to equilibrate for 1 hour, washed with fresh culture medium, and suspended as 0.04 g tissue per 1 mL medium into 48-well plates (BD Falcon) in a total volume of 0.5 mL per well. Medium containing 10 mg/mL IL-4, IL-5, or IL-13 (PeproTech, NJ, USA) was added to wells and the nasal polyp fragments were incubated for 24 hours at 37°C. Tissue fragments cultured with 0.5 mL of TCM medium alone were used as controls. Supernatant was collected from each well, and the tissue pellets were snap frozen and processed further for determination of MUC5AC and MUC5B.

2.5 | Real-time RT-PCR

Real-time RT-PCR and data analysis were performed using 7500 Real-time PCR System (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. Total RNA was extracted from the dissociated tissues, using TRNzol Reagent (TianGen Co.Ltd, Beijing, China). Reverse transcription was performed using PrimeScriptTM RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China). One microgram of total RNA was reverse transcribed in a total volume of 10 μ L. Expression of IL-4R α mRNA was normalized to RNA loading for each sample using GAPDH mRNA as an internal standard, primers (sense, 5'-CCTCTGACTTCAACAGCGACAC-3'; antisense, 5'-TGGTCCAGGG GTCTTACTCC-3'). IL4R α primers (sense, 5'-GCAGTGGCATTGTCT ACTCAGC-3'; antisense 5'-GGTGTAGGGGGCGAGGA-3') were chosen to amplify a 149 bp fragment. PCR conditions for amplification of

Mediators/Mucins	Control (pg/mL)	CRSwNP (pg/mL)	P value
IL-5	9.8 (7.6-9.9)	284 (11.9-649.8)***	.000
IL-13	59.3 (26.8-65.5)	102.4 (51.2-386.8)*	.028
IL-4	5.5 (5.5-19.8)	77 (19.8-126.5)**	.001
IFN- γ	22.1 (22.1-37.7)	22.1 (22.1-166.9)	.324
IL-17	16.5 (16.3-72.4)	16.4 (16.4-16.6)	.401
ECP	95.4 (15.3-608.0)	4023.6 (2131.2-13557.5)***	.000
IgE	20.2 (9.1-121.7)	191.2 (80.1-407.2)**	.005
MPO	2511.5 (1096.9-4161.9)	3449.6 (1727.3-6783.0)	.349
MCP-1	3686.5 (3049.7-4829.4)	4916.1 (3199.5-6061.4)	.279
MUC5AC	30.1 (19.0-58.1)	227.9 (114.0-433.3)***	.000
MUC5B	170.7 (93.4-223.1)	1152.9 (689.2-1915.7)***	.000

Data are expressed as median and interquartile range. P values signs after Mann-Whitney U test means as follow: * $P < .05$, ** $P < .01$, *** $P < .01$ in Control vs CRSwNP.

IL-4R α were 45 cycles at 95°C for 5 seconds, 60°C for 40 seconds, followed by melting curve analysis. Quantification of transcript level of IL-4R α gene was conducted according the $2^{-\Delta\Delta C_t}$ method.

2.6 | Statistical analysis

Statistical analysis was performed using SPSS 20.0 software. Categorical variables were compared using Fisher's exact test. The Mann-Whitney U test was applied to evaluate the statistical significance of differences in the expression of cytokines and mucins between the 2 patient groups. Data comparison within different patient groups was performed using the Kruskal-Wallis test. Correlations between mediators associated with T helper cytokines and MUC5AC, MUC5B in CRSwNP, were calculated using Spearman correlation coefficient. Values of $P < .05$ were considered to be significant.

3 | RESULTS

3.1 | Expression of MUC5AC, MUC5B and levels of inflammatory markers in nasal tissue

Concentrations of IL-5, IL-13, IL-4, MUC5AC, and MUC5B were significantly increased in the CRSwNP group, compared to the control group (Table 2). Concentrations of IFN- γ and IL-17 were not significantly different between control and CRSwNP groups. Based on cytokine and mediator measurements, particularly presence or absence of IL-5, we divided the CRSwNP samples into 2 endotypes: IL-5(+) CRSwNP ($n = 15$) and IL-5(−) CRSwNP ($n = 6$) for further analysis. Assessment of mucin expression in these endotypes of CRSwNP defined according to Th inflammatory patterns showed that IL-5(+) CRSwNP samples ($n = 15$) had significantly increased expression of MUC5AC ($P < .01$ and $P < .001$, respectively; Figure 1A) and MUC5B (both $P < .001$; Figure 1B), compared to IL-5(−) CRSwNP samples and control samples. Expression of MUC5AC and MUC5B was also higher in IL-5(−) CRSwNP samples than in controls. Similarly, the levels of IL-4, IL-13, IgE, and ECP; but not IFN- γ or IL-17; were significantly increased in IL-5(+)

TABLE 2 Cytokines, mediators, and mucins measured in nasal tissue homogenates

CRSwNP samples compared with IL-5(−) CRSwNP and control samples (Figure 1C,D).

3.2 | Correlation between mediators associated with T helper cytokines and MUC5AC, MUC5B in CRSwNP

Different Th cytokine inflammatory environments affect the function of related inflammatory mediators. We measured the levels of ECP, IgE, MPO, and MCP-1 and analyzed the correlation between these mediators associated with Th cytokines and MUC5AC, MUC5B in CRSwNP. We found that levels of both MUC5AC and MUC5B were significantly correlated with levels of IgE (Spearman correlation coefficient .662, $P = .001$; .640, $P = .002$) and ECP (Spearman correlation coefficient .518, $P = .016$; .583, $P = .006$) in CRSwNP tissues. No correlation was found between MUC5AC or MUC5B and MPO, MCP-1 in CRSwNP (Figure 2A-H).

3.3 | Expression of MUC5AC and MUC5B in nasal polyp tissue fragments stimulated by Th2 cytokines

To clarify the role of Th2 cytokines in mucin expression, we measured the expression of MUC5AC and MUC5B in cultured nasal polyp tissue fragments stimulated with or without IL-5 (10 ng/mL), IL-13 (10 ng/mL), or IL-4 (10 ng/mL) for 24 hours. IL-14 and IL-13 significantly increased the secretion of both MUC5AC (both $P < .01$, $n = 6$; Figure 3A) and MUC5B ($P < .01$ and $P < .05$, respectively, $n = 6$; Figure 3B) compared to TCM controls. In contrast, IL-5 did not significantly alter the secretion of MUC5AC or MUC5B compared with TCM controls.

3.4 | Expression and localization of IL-4 receptor alpha (IL-4R α) in nasal polyps from CRSwNP

To further study the potentially direct effect of IL-4 or IL-13 on mucin secretion, we examined localization of IL-4R α , MUC5AC, and MUC5B in nasal tissue from control subjects (Figure 4A,C,E) and nasal polyps

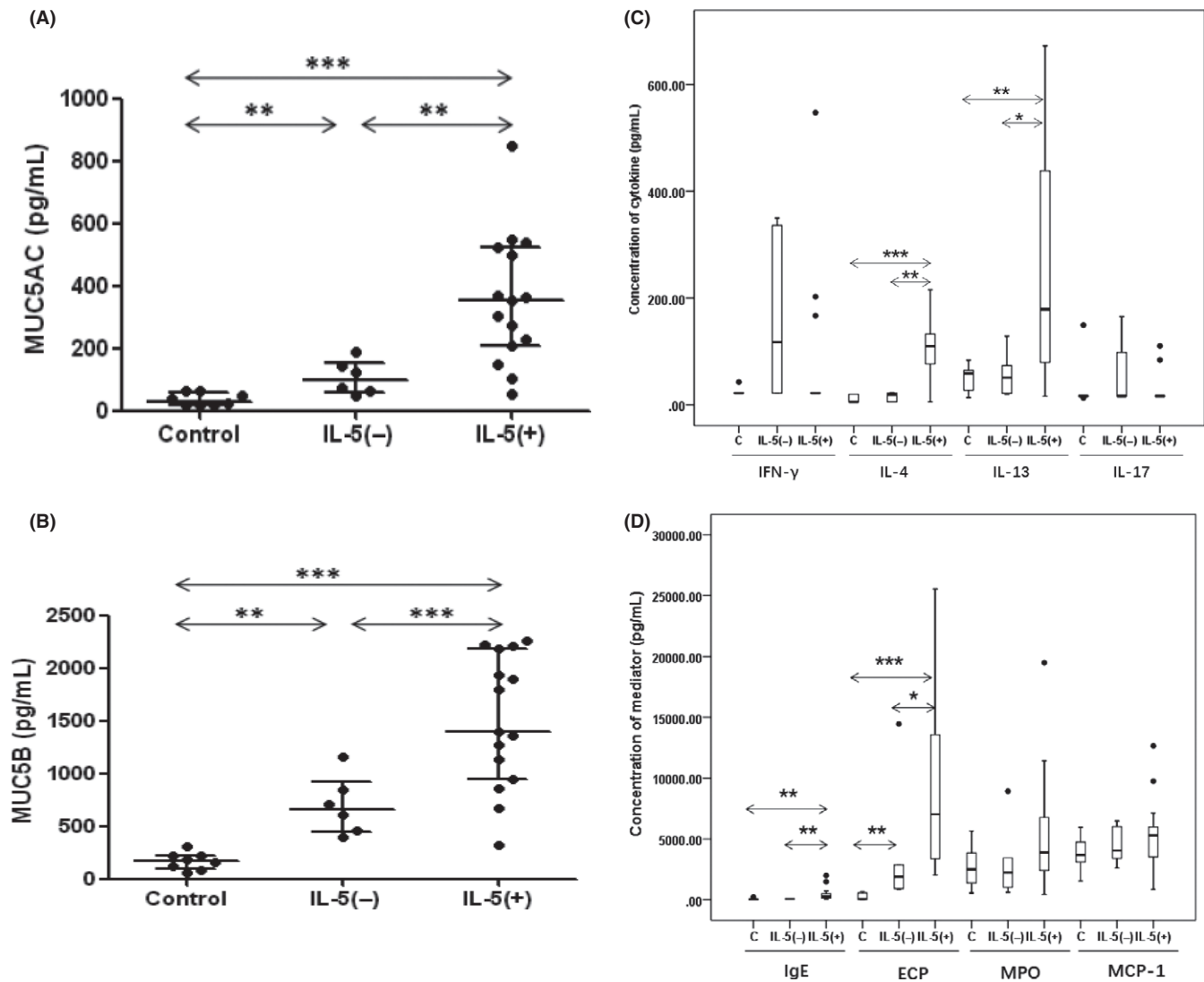


FIGURE 1 Measurement of MUC5AC (A), MUC5B (B), IFN- γ , IL-4, IL-13, IL-17 (C), IgE, ECP, MPO, and MCP-1 (D) by ELISA in controls (n = 8), IL-5(+) (n = 15), and IL-5(-) (n = 6) nasal polyp samples from CRSwNP patients. Values represent median and interquartile range. * $P < .05$, ** $P < .01$, *** $P < .001$

from CRSwNP patients (Figure 4B,D,F). Immunohistochemical analysis of nasal tissue serial sections revealed that IL-4R α was localized in similar areas with MUC5AC(+) and MUC5B(+) cells in epithelium and sub-mucosal glands in both control and nasal polyp tissues from CRSwNP. Furthermore, IL-4R α was also present in various sub-epithelial cells such as endothelial cells and mesenchymal cells (Figure 4A-F). Furthermore, real-time RT-PCR demonstrated that expression of IL-4R α mRNA was significantly higher in IL-5(+) CRSwNP samples compared to control samples ($P < .001$) and IL-5(-) CRSwNP samples ($P < .05$). Similarly, expression of IL-4R α was significantly higher in IL-5(-) CRSwNP samples than in control samples ($P < .01$) (Figure 4G).

4 | DISCUSSION

Different inflammatory features are embodied in CRSwNP biased by T helper (Th) cytokines. However, it remains to be determined

whether Th cytokines dominant in CRSwNP are involved in mucin upregulation. In this regard, to our knowledge, the present study is the first to systematically report the relationship between the inflammatory pattern and mucin over-secretion in different endotypes of CRSwNP classified by expression of Th cytokines.¹⁹ We found that increased protein levels of IL-4, IL-5, and IL-13 were detected in nasal polyp tissue from CRSwNP patients, in comparison with normal nasal mucosa. Th2 associated inflammatory mediators, such as IgE and ECP, were also significantly correlated with expression of MUC5AC and MUC5B. Moreover, expression of MUC5AC, MUC5B, IL-4, and IL-13 was significantly increased in IL-5(+) CRSwNP. IL-4 and IL-13, but not IL-5, induced overexpression of MUC5AC and MUC5B in cultured nasal fragments. So our further investigation to examine the localization and expression of IL-4R α in the nasal tissue demonstrated that IL-4R α was widely expressed in various cells, including goblet cells from epithelium and mucous cells from sub-mucosal glands, and was increased significantly in IL-5(+) CRSwNP.

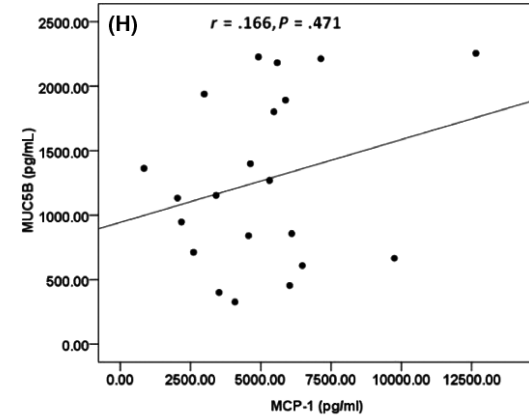
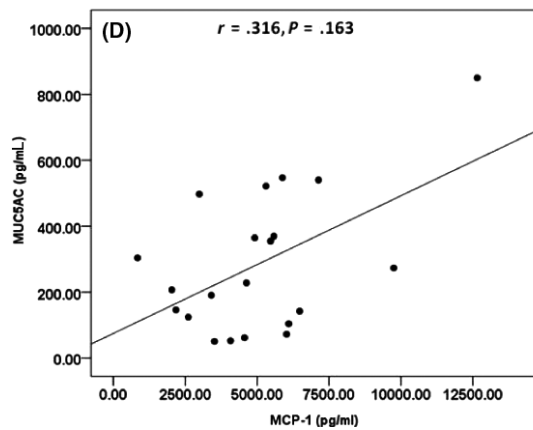
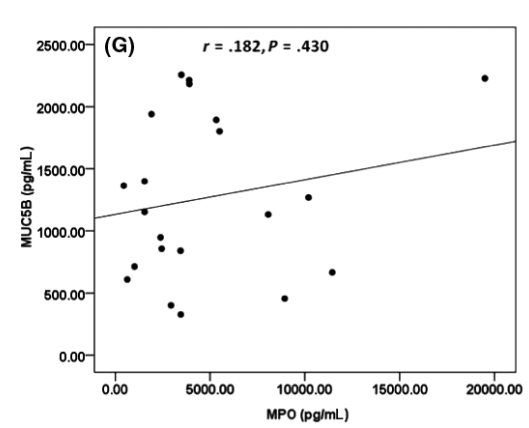
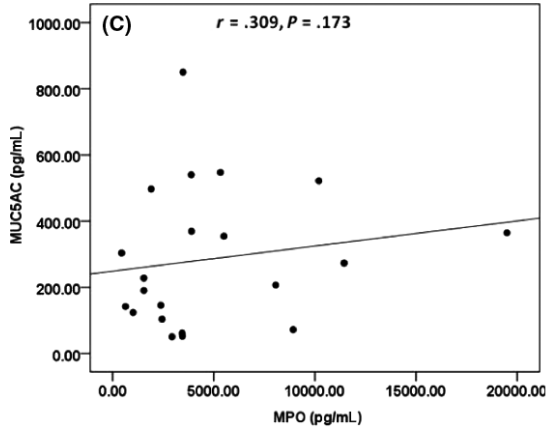
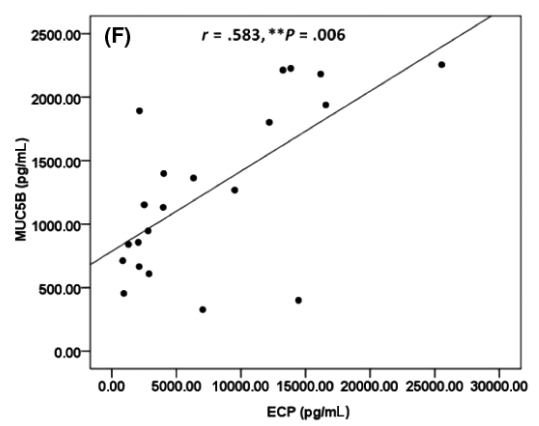
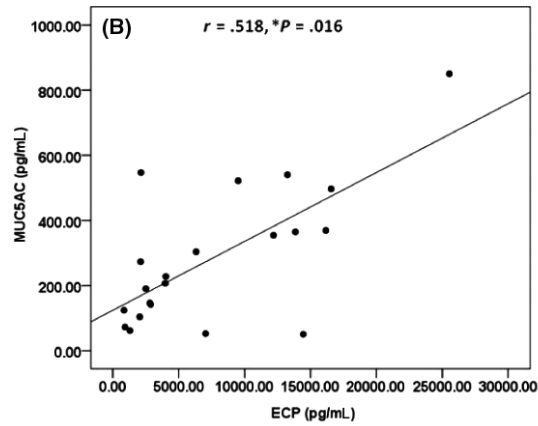
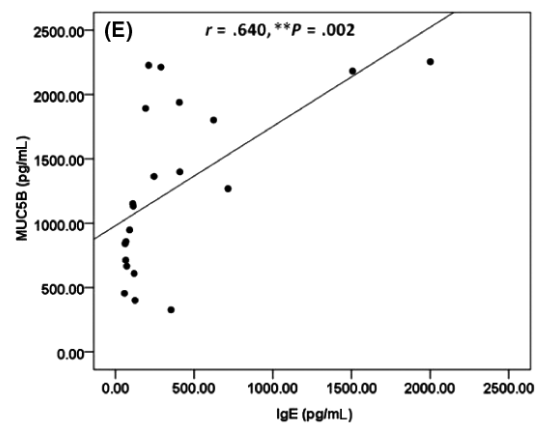
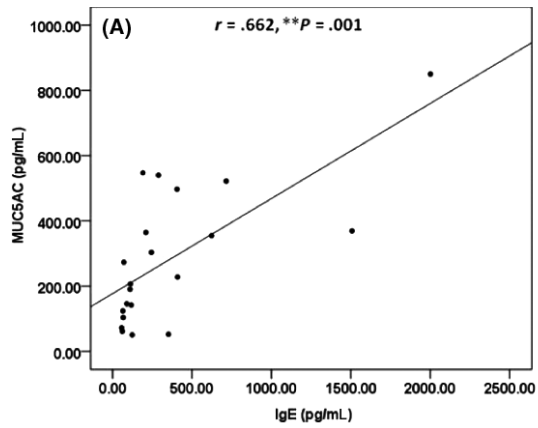


FIGURE 2 Scatter plots showing correlations between MUC5AC, or MUC5B and other mediators associated with T helper cytokines (Spearman's rho) in CRSwNP. The correlation between MUC5AC and IgE (A), ECP (B), MPO (C), MCP-1 (D); the correlation between MUC5B and IgE (E), ECP (F), MPO (G), MCP-1 (H). ** $P < .01$, * $P < .05$

Classification of endotypes in chronic airway diseases has received increasing attention over the last few years, and these are defined by distinct pathophysiologic mechanisms that might be identified by corresponding biomarkers.²⁰ Mucous metaplasia, as one of most important features of tissue remodeling in CRSwNP, has been shown in previous studies.^{5,21} However, the level and possible mechanism/s underlying mucus metaplasia are little known in different endotypes of CRSwNP, which are mostly classified on the basis of the expression of inflammatory biomarkers. Depending on the sub-classification of CRSwNP based on the presence of mostly eosinophils or neutrophils, Ikeda et al²² found that the expression of MUC5AC was greater in the eosinophilic CRSwNP group than in both the neutrophilic and non-eosinophilic non-neutrophilic groups. More recently, Du et al²³ have demonstrated that the expression of MUC5AC and MUC5B, as well as goblet cells hyperplasia, was

significantly elevated in IL-5(+) nasal polyps. Furthermore, these changes in IL-5(+) nasal polyps were correlated with the decreased expression of airway epithelial-specific transcription factor NK2 homeobox 1 (NKX2-1). In the present study, we went investigated the mechanism of mucin over-secretion in the different endotypes of CRSwNP from the perspective of Th cytokine inflammatory patterns and found that the expression of MUC5AC and MUC5B was upregulated in nasal polyps from CRSwNP, especially IL-5(+) CRSwNP, and correlated with IgE and ECP associated with Th2 cytokines. Our study showed that the levels of IL-13 and IL-4 were significantly increased in IL-5(+) CRSwNP, compared to IL-5(−) CRSwNP and control group. Although the expression of MUC5AC and MUC5B was also increased in IL-5(−) CRSwNP, the Th2 cytokines did not appear to play a role in the process of metaplasia. Whether other Th cytokines are involved in mucin over-secretion in IL-5(−), CRSwNP needs to be studied further.

IL-4/IL-13 signaling is mediated via the type II IL-4 receptor on cells of non-hematopoietic stem cell origin, which binds both IL-4 and IL-13 and is comprised of IL-4R α and IL-13R α 1.²⁴ IL-4R α plays a key role in airway inflammation and tissue remodeling by binding with IL-4 directly or enhancing the affinity of IL-13 with IL-13R α 1.^{25,26} Presently, there are only a few reports on the effect of IL-4R α expression on mucin secretion in CRSwNP. A recent trial with Dupilumab, an anti-IL4R α mAb, showed a clinically highly relevant effect on nasal polyp size and symptoms, including mucus secretion.²⁷ To investigate the mechanism of downstream signaling of IL-4 and IL-13 on mucin over-secretion, we assessed the location and expression of IL-4R α in nasal tissues. Our study showed that IL-4R α was localized in similar areas with goblet cells and mucous cells from submucosal glands and for the first time demonstrated high IL-4R α expression in nasal tissues from IL-5(+) CRSwNP; indicating that IL-4R α was likely to participate in the regulation of mucin overexpression in IL-5(+) CRSwNP. Although we did not find the differences in the levels of the main Th cytokines between IL-5(−) CRSwNP and control, whether the increase in IL-4R α expression can lead to mucin over-secretion in IL-5(−), CRSwNP needs to be investigated further.

The existence of a predominantly inflammatory environment in these chronic airway diseases might influence mucin expression dramatically. Although some studies have reported that IL-17 might stimulate MUC5AC or MUC5B gene expression in airway epithelial cells,^{28,29} the lack of any significant differences in the levels of IL-17 measured between control and CRSwNP group, and the generally low level of IL-17 detected in the nasal tissues, suggest that IL-17 is unlikely to influence the expression of mucin in CRSwNP. In contrast, the present study has demonstrated that the expression of IL-4, IL-13, and IL-4R α is increased significantly in IL-5(+) CRSwNP and that MUC5AC and MUC5B secretion could be induced by IL-4 and IL-13, but not IL-5, in cultured nasal tissue fragments in vitro. IL-5

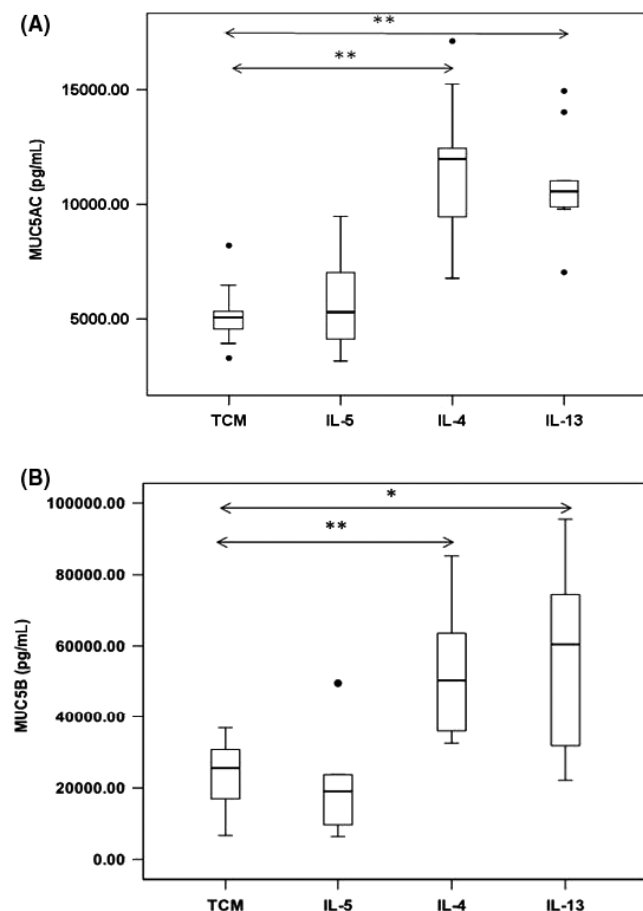


FIGURE 3 Measurements of MUC5AC (A) and MUC5B (B) by ELISA in the supernatants of nasal polyp tissue fragments from CRSwNP patients incubated with TCM, IL-5 (10 ng/mL), IL-4 (10 ng/mL), and IL-13 (10 ng/mL) for 24 h ($n = 6$). All values represent median and interquartile range. * $P < .05$, ** $P < .01$

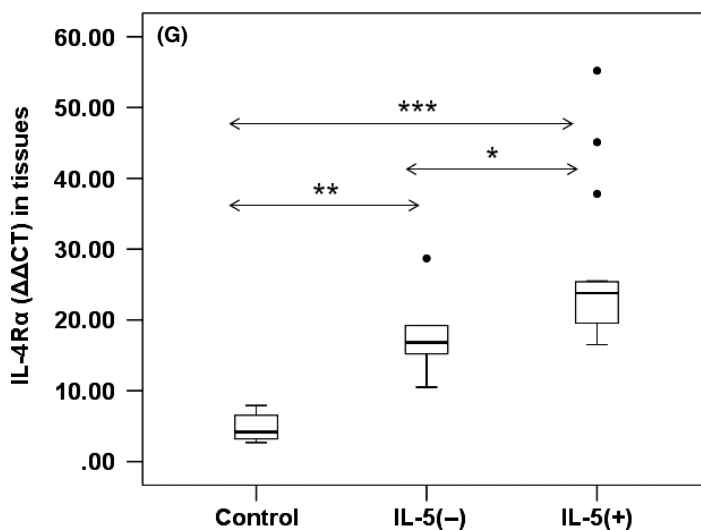
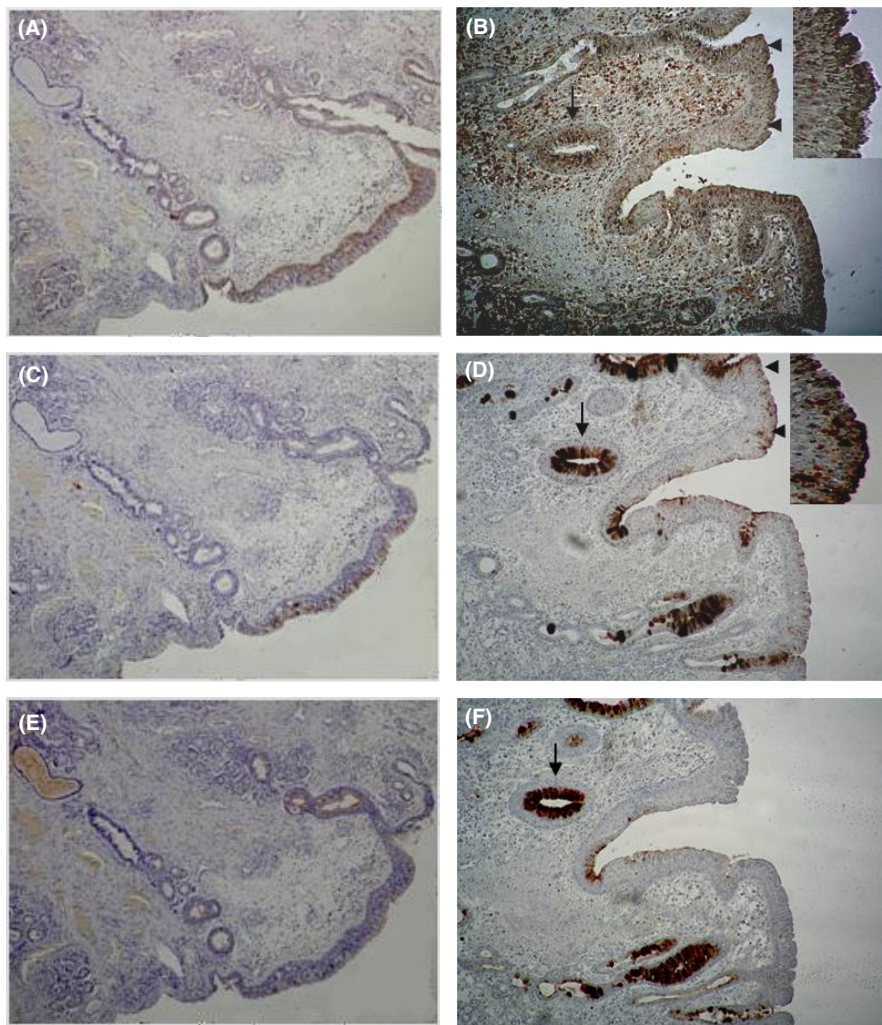


FIGURE 4 Localization of IL-4 receptor alpha (IL-4R α), MUC5AC and MUC5B in control (A, C, E) and CRSwNP groups (B, D, F) by immunohistochemistry. Positive staining of IL-4R α , MUCAC, and MUC5B was seen in cells from epithelium (arrowheads) and submucosal glands (arrows) of nasal polyp tissues. All sections were observed by light microscopy at $\times 200$ magnification. Expression of IL-4R α in tissues from control tissues ($n = 8$), and IL-5(-) ($n = 6$) and IL-5(+) ($n = 15$) nasal polyp samples from CRSwNP patients, as demonstrated by real-time RT-PCR (G). Data are shown as mean $\pm$ SEM values for comparative cycle time ratios (CT) for IL-4R α normalized to GAPDH. * $P < .05$, ** $P < .01$, *** $P < .001$

plays an important role in eosinophil recruitment and activation and activated eosinophils can induce goblet cell metaplasia.^{30,31} Indeed, it has been demonstrated that addition of IL-3+ GM-CSF or IL-3+ IL-5 to eosinophils cultured with NCI-H292 cells leads to increased production of MUC5AC from these cultures.³² However, although IL-5 could not stimulate mucin expression directly in vitro, significant

correlation of ECP with MUC5AC and MUC5B in nasal polyps from CRSwNP suggests that the inflammatory environment established by high levels of IL-5 in vivo, including high levels of Th2 cytokines and other inflammatory mediators, might have a role in goblet cell metaplasia and mucin overexpression. In the present study, we did not differentiate endotypes of CRSwNP in the

stimulation test in vitro, because we wanted to mainly verify the direct effect of Th2 cytokines on mucin expression in nasal polyps.

In summary, we have found that the Th2 inflammatory pattern is closely related to the expression of MUC5AC and MUC5B. Furthermore, high levels of IL-4, IL-13, and their common receptor IL-4R α expressed on mucus secreting cells may directly induce mucin over-secretion in IL-5(+) CRSwNP. This study highlights an important function of Th2 cytokines as regulators of mucin expression and may provide new strategies for modulating mucus over-secretion, as shown by anti-IL4R α antagonism.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Yu Zhang, Lara Derycke, and Gabriele Holtappels performed practical experiments. Yu Zhang and Luo Zhang wrote the first draft of the article. Xiangdong Wang, Luo Zhang, Claus Bachert, and Nan Zhang designed the study and contributed to the interpretation of the data and statistics.

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