

## Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease

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**Background:** Chronic rhinosinusitis with nasal polyps (CRSwNP) is characterized by the accumulation of inflammatory cells; however, an eosinophil predominance is seen in white (Belgian), but not Asian (south Chinese), patients with polyps.

**Objective:** We sought to investigate the association of inflammatory cell predominance with regulatory T-cell and T-effector cell patterns.

**Methods:** Nasal mucosal tissue was obtained from 26 consecutive Belgian patients with CRSwNP and 21 Belgian control subjects and 29 south Chinese patients with CRSwNP and 29 south Chinese control subjects, who all underwent phenotyping, including nasal endoscopy and computed tomographic scanning. Tissues were investigated for granulocytes and their products and T-effector/regulatory T cells and related cytokines.

**Results:** Both CRSwNP groups were comparable in terms of symptoms, computed tomographic scan results, and nasal endoscopy results, but asthma comorbidity was significantly higher in white patients. Tissue from white patients with CRSwNP was characterized by eosinophilic inflammation (eosinophil cationic protein/myeloperoxidase ratio > 2), whereas samples from Asian patients were biased toward neutrophilic inflammation (eosinophil cationic protein/myeloperoxidase ratio = 0.25). Both CRSwNP groups demonstrated significant upregulation of the T-cell activation marker soluble IL-2 receptor  $\alpha$  and significant downregulation of Foxp3 expression and TGF- $\beta$ 1 protein content versus their respective control groups. However, whereas white patients displayed a significant increase in T<sub>H</sub>2 cytokine and related

marker levels versus control subjects and versus Asian patients, the latter showed a T<sub>H</sub>1/T<sub>H</sub>17 cell pattern versus control tissue. **Conclusion:** Nasal polyps (CRSwNP) from white and Asian patients are both characterized by T-cell activation and impaired regulatory T-cell function; however, T-effector cells in the samples from white patients were T<sub>H</sub>2-biased, whereas samples from their Asian counterparts demonstrated a T<sub>H</sub>1/T<sub>H</sub>17 polarization. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

**Key words:** Airway inflammation, chronic rhinosinusitis, nasal polyps, eosinophils, T<sub>H</sub>2, T<sub>H</sub>17, regulatory T cells, IL-17A, TGF- $\beta$ 1, Foxp3

Chronic rhinosinusitis with nasal polyps (CRSwNP), a subgroup of chronic rhinosinusitis manifestations, clinically is characterized by the appearance of nasal polyps (NPs) in the nasal cavity that are visible to the physician through nasal endoscopy; they cause typical symptoms, such as nasal obstruction, loss of smell, anterior and posterior secretion, and a diffuse occupancy of the paranasal sinuses recognized on computed tomographic (CT) scanning.<sup>1,2</sup> As has been described before in European and US studies, histology would reveal prominent edema formation and tissue eosinophilia in the vast majority of polyp specimens, the latter being even more pronounced in patients with concomitant asthma, aspirin sensitivity, or both.<sup>3,4</sup> CRSwNP in white subjects has consistently showed a T<sub>H</sub>2 polarization with high IL-5 and IgE concentrations and low levels of TGF- $\beta$ 1.<sup>1</sup> Of note, NPs in patients with cystic fibrosis show edema formation and matrix disruption also but display a prominent neutrophilic instead of eosinophilic inflammation and a significantly lower tissue eosinophil cationic protein (ECP) concentration compared with NPs from patients with CRSwNP, suggesting that edema formation might not necessarily be dependent on tissue eosinophils and their activation.<sup>5</sup>

Eosinophils in patients with CRSwNP are activated and survival is increased by IL-5–induced reduction of apoptosis,<sup>6,7</sup> and these eosinophils are accumulated within the tissue by eosinophil-specific adhesion receptors and chemokines, such as eotaxin.<sup>8,9</sup> Those cytokines, and consequently the eosinophilic inflammation, are further increased in polyps with specific IgE to *Staphylococcus aureus* enterotoxins, which serve as superantigens, induce a polyclonal T-cell and B-cell activation with IgE formation, and amplify the eosinophilic inflammation.<sup>10,11</sup> Consistent with the edema formation in NPs, an upregulation of matrix metalloproteinase 7 and 9, but not of tissue inhibitor of metalloproteinases 1, has been described, which is in line with

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**Abbreviations used**

Be NP:	Nasal polyps from Belgian patients
Be CO:	Control tissue from Belgian patients
CRSwNP:	Chronic rhinosinusitis with nasal polyps
CT:	Computed tomography
ECP:	Eosinophil cationic protein
Foxp3:	Forkhead box P3
MPO:	Myeloperoxidase
NP:	Nasal polyp
RORC:	Retinoic acid–related orphan receptor C
S-Ch CO:	Control tissue from south Chinese patients
S-Ch NP:	Nasal polyps from south Chinese patients
STAT:	Signal transducer and activator of transcription
T-bet:	T-box transcription factor

a lack of upregulation of TGF- $\beta$ 1, a tissue inhibitor of metalloproteinases 1–inducing and profibrotic growth factor.<sup>5</sup>

Recent studies in patients with CRSwNP from South China, however, have suggested that clinically equivalent NP disease also might exist with poorly expressed eosinophilia and a lack of IL-5 and eotaxin expression in the tissue,<sup>12</sup> as was described earlier in Thai patients.<sup>13</sup> Histologically, these polyps still showed comparable edema formation, as seen in their white counterparts, and also were characterized by an increase in T and plasma cells.<sup>12</sup>

The analysis of common and distinct pathophysiologic features in NPs from Asian versus white patients might help to clarify key cytokines of the inflammatory processes and consequently therapeutic targets. It is therefore of great interest to further characterize the inflammatory granulocytes, the orchestrating T<sub>H</sub>1/T<sub>H</sub>2/T<sub>H</sub>17 cell polarization, and the regulatory T-cell status in both disease groups in terms of cytokine profiles and transcription factors. As described before, we used mRNA levels of GATA-3, T-box transcription factor (T-bet), retinoic acid–related orphan receptor C (RORC), and Foxp3 and protein levels of IL-5, IFN- $\gamma$ , and IL-17A in tissue homogenates as a surrogate for T<sub>H</sub> subpopulations.<sup>14</sup>

**METHODS****Patients**

Nasal tissue was obtained from 26 Belgian patients with CRSwNP and 21 Belgian control patients at the department of Oto-Rhino-Laryngology of the University Hospital of Ghent, Belgium (Be NP and Be CO). A second group of patients with CRSwNP ( $n = 29$ ) and control patients ( $n = 29$ ) was included at the Zhongshan Hospital in Guangdong province, South China, using identical standard operating procedures (S-Ch NP and S-Ch CO). NP samples were obtained during routine endonasal sinus surgery in consecutive patients scheduled for surgery unrelated to the study. Patients undergoing septoplasty because of anatomic variations were considered control subjects, and samples were obtained from the inferior turbinate during septal surgery. The diagnosis of sinus disease was based on history, clinical examination, nasal endoscopy, and CT scanning of the sinuses according to the European Position Paper on Rhinosinusitis and Nasal Polyps guidelines.<sup>1</sup> CT scans were graded according to the Lund-MacKay method.<sup>15</sup> Individual rhinosinusitis symptoms were evaluated by a physician on a scale of 0 to 3 (no symptoms, mild symptoms, moderate symptoms, and severe symptoms) before surgery. Polyps were graded by size and extent in both the left and right nasal fossa on a scale of 0 to 3, according to the Davos classification.<sup>1</sup> The reasons for surgical procedures were unrelated to the study in all patients.

The atopic status was evaluated by using the Phadiotop test (Phadia, Uppsala, Sweden), detecting IgE antibodies against various common inhalant

allergens. The diagnosis of asthma and aspirin sensitivity was performed by a pneumologist based on lung function and challenge tests, where applicable. None of the subjects used oral or nasal corticosteroids 4 weeks before surgery or antibiotics within the last 2 weeks. All patients provided written informed consent, and the ethics committees of the Ghent University Hospital and the Zhongshan Hospital China approved the study.

**Immunohistochemistry**

Paraffin sections were stained with mouse mAbs for myeloperoxidase (MPO; clone 2C7; Serotec, Oxford, United Kingdom), as previously described.<sup>5</sup> Edema and eosinophil counts were evaluated on hematoxylin and eosin–stained sections. Edema was semiquantitatively scored in 10 serial sections on a 3-point scale, with 0 representing the lowest and 2 representing the highest scores. The scoring system was calibrated by examining a representative number of samples. The number of positive cells was analyzed in 10 fields by using a magnification of  $\times 400$  and scored by 2 independent observers who did not know the diagnosis and clinical data, as previously described.<sup>12</sup>

**Measurement of cytokine and IgE levels in tissue homogenates**

Freshly obtained tissue specimens were homogenized, as previously described,<sup>12</sup> and assayed for eotaxin, soluble IL-2 receptor  $\alpha$ , IL-5, IFN- $\gamma$ , IL-10, IL-1 $\beta$ , IL-6, IL-17A, TGF- $\beta$ 1, and MPO by using commercially available ELISA kits (Quantikine ELISA, R&D Systems, Minneapolis, Minn; MPO, Oxis International, Portland Ore). IgE and ECP were measured by using the UNICAP system (Pharmacia, Uppsala, Sweden). An ECP/MPO ratio was calculated.

**Quantitative real-time PCR**

mRNA levels of the transcription factors GATA-3, Foxp3, RORC, and T-bet were determined by means of real-time PCR, as in previous publications.<sup>16</sup> The expression of 3 housekeeping genes,  $\beta$ -actin, hydroxymethylbilane synthase, and elongation factor 1, was used to normalize for transcription and amplification variations among samples after a validation with geNorm software (Ghent University, Ghent, Belgium).<sup>16</sup> The relative expression units of each gene per 20 ng of cDNA sample was determined by using the qBase program (version 1.3.5; Ghent University, Ghent, Belgium), which consists of a collection of Microsoft Excel sheets that automatically analyze real-time quantitative PCR data, combining the  $\Delta$ CT relative quantification model with PCR efficiency correction and multiple reference gene normalization.

**Statistical analysis**

Statistical analysis was performed with Graphpad Prism software and SPSS 11.0 (SPSS, Inc, Chicago, Ill). Data are expressed in bar charts that represent medians and interquartile ranges. Statistical analysis was performed by using the Kruskal-Wallis test and the Mann-Whitney  $U$  2-tailed test for unpaired comparisons. When comparisons were made between groups, the Kruskal-Wallis test was used to establish the significant intergroup variability. The Mann-Whitney  $U$  test was then used for between-group comparisons. Baseline variables were analyzed by using a 1-way ANOVA test or the Fisher exact test. The significance level was set at an  $\alpha$  value of .05.

**RESULTS****Patient characteristics**

Clinical characteristics, disease-specific symptom scores, and IgE data of all patients are summarized in Table I. Groups were comparable in terms of age, female/male ratio, and atopy (positive Phadiotop result). S-Ch NPs and Be NPs both shared a significantly higher total symptom score, polyp score in the nasal cavity determined by means of endoscopy, and CT score, demonstrating

**TABLE 1.** Patient characteristics and symptom scores

	South Chinese control subjects	South Chinese patients with NPs	Belgian control subjects	Belgian patients with NPs	ANOVA (Fisher exact test*)
No.	29	29	21	26	
Age (y), median (range)	38.6 (33.2-43.5)	36.4 (28.6-46.5)	30.3 (21.3-37.9)	46.2 (38.4-55.5)	
Female/male sex	10/19	9/20	9/12	11/15	.767
Asthma	0/29	2/29	2/21	14/26	<.0001*†‡
Positive Phadiotop result	11/29	9/29	8/21	11/26	.845
Aspirin intolerance	0/29	0/29	0/21	7/26	<.0001*†‡
CT score (Lund-Mackay)	0	16 (11-20)	1 (0-2)	13 (11-20)	<.0001†‡
Polyp score (Davos)	0 (0-0)	5 (4-6)	0 (0-0)	4 (4-6)	<.0001†‡
Total symptom score	5 (3-6)	10 (7-11)	5 (3-7)	9 (7-11)	<.0001†‡
Nasal congestion	2 (2-3)	3 (2-3)	2 (1-3)	3 (2-3)	.033†
Rhinorrhea	0 (0-1)	2 (1-3)	0 (0-2)	1 (0-2)	.008†
Sneezing	0 (0-1)	1 (0-2)	0 (0-2)	0 (0-1)	.093
Loss of smell	0 (0-1)	2 (2-3)	0 (0-1)	3 (2-3)	<.0001†‡
Headache	1 (0-2)	2 (1-3)	1 (0-2)	2 (1-2)	.006†‡

The total symptom score is the sum of the scores for nasal congestion, rhinorrhea, headache, postnasal drip, loss of smell, and sneezing. Data are expressed as medians and interquartile ranges. The level of significance (*P*) was obtained by means of ANOVA or the Fisher exact test (marked with \*). For comparisons of continuous variables between 2 groups, the Mann-Whitney *U* test was performed.

†Comparison between S-Ch COs and S-Ch NPs.

‡Comparison between Be COs and BE NPs. There was no significant difference between Be NPs and S-Ch NPs. The significance level was set at an  $\alpha$  value of .05.

sinus involvement compared with those seen in the respective control subjects. This also was reflected in the individual scores for nasal congestion, rhinorrhea, and loss of smell. Thus the typical disease characteristics of NPs were equivalent, with S-Ch NPs showing a slightly higher symptom, CT, and endoscopic score compared with Be NPs. However, asthma was significantly more frequent in the Be NPs compared with that seen in Be COs and S-Ch NPs, and aspirin intolerance was only noted in Be NPs.

### Histology, cellular pattern, and granulocyte markers

Further information on histology, cellular pattern, and granulocyte markers is shown in Fig 1. Both NP groups scored significantly higher than their respective control subjects and comparably with each other for tissue edema and showed the typical pseudocyst formations and extracellular matrix disruptions. Eosinophil (hematoxylin stain) and neutrophil (MPO) counts were significantly higher in the NP groups compared with those in their respective control subjects but also showed significantly higher numbers in Be NPs versus S-Ch NPs.

Apart from their presence, granulocytes were assayed for their activity by measuring ECP and MPO concentrations in the tissue. Whereas ECP concentrations were significantly higher in Be NPs versus Be COs and S-Ch NPs, MPO concentrations only were increased in Be NPs versus Be COs. However, the ECP/MPO ratio reflecting the dominance of a subgroup of granulocytes was 2.08 in Be NPs but 0.25 in S-Ch NPs, evidencing an eosinophil bias in Be NPs versus a neutrophil bias of inflammation in S-Ch NPs.

### Real-time PCR

Further information on real-time PCR results is shown in Fig 2. Expression of mRNA for GATA-3 was significantly upregulated in Be NPs versus Be COs and S-Ch NPs but not in S-Ch NPs versus S-Ch COs, whereas mRNA for T-bet was significantly overexpressed in Be NPs and S-Ch NPs versus that seen in their respective control tissues. Foxp3 expression was significantly downregulated in both polyp groups versus that seen in their

respective control subjects, whereas there were no significant differences of RORC expression.

### Mediators, cytokines, and IgE in tissue homogenates

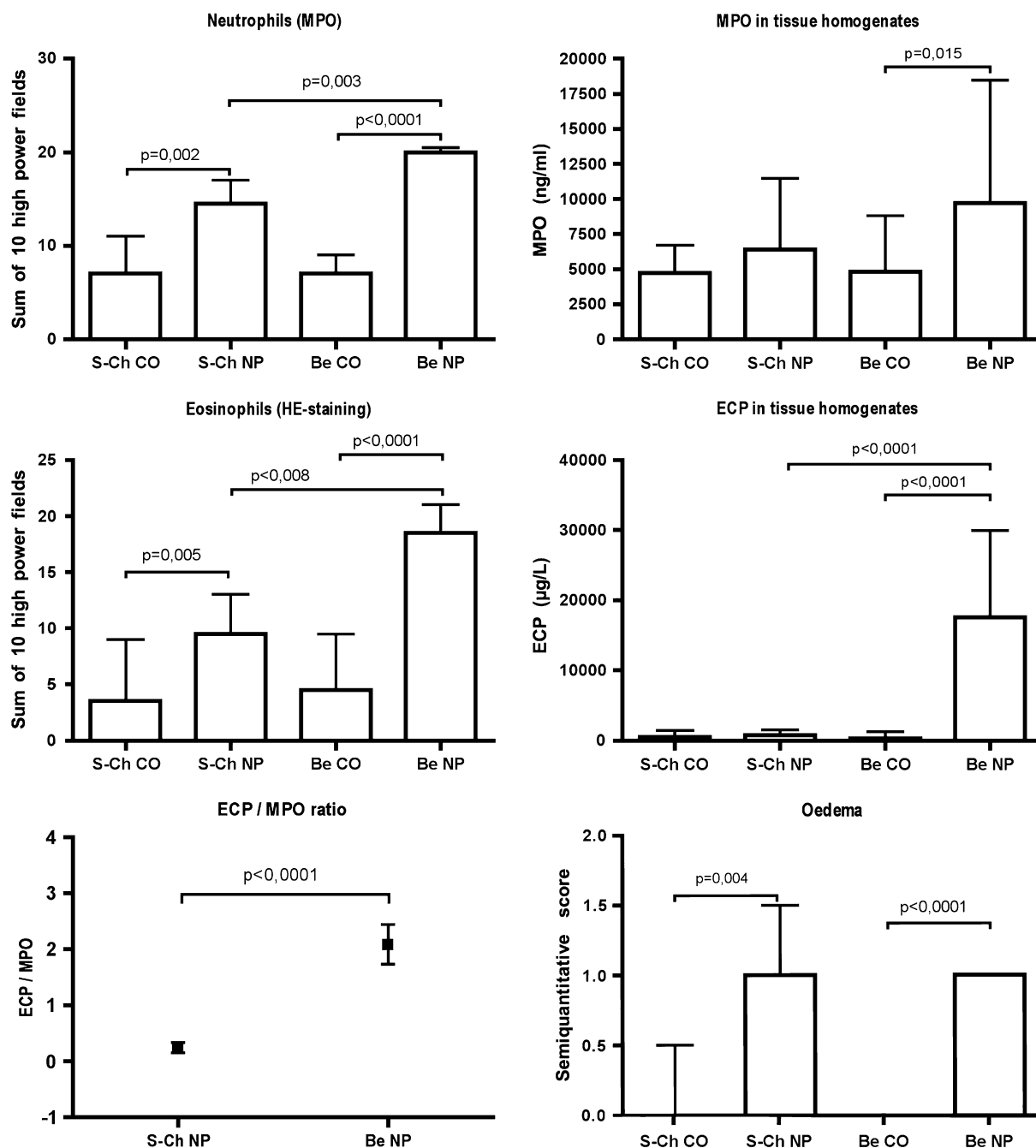
Further information on mediators, cytokines, and IgE in tissue homogenates is shown in Fig 3. Soluble IL-2 receptor  $\alpha$ , used as a marker for T-cell activation, was significantly increased in both NP groups versus levels seen in corresponding control groups, although a difference could also be noted between the control groups. ECP, eotaxin, IL-5, and total IgE levels in the tissue were significantly and impressively increased in Be NPs versus S-Ch NPs and Be COs, whereas S-Ch NPs did not demonstrate any significant change in levels of those proteins versus S-Ch COs. In clear contrast, IFN- $\gamma$ , IL-1 $\beta$ , IL-6, and IL-17 protein levels were significantly upregulated in S-Ch NPs versus S-Ch COs and Be NPs, whereas such upregulation was not seen in Belgian samples.

In Be NPs, but not in S-Ch NPs (because of a lack of IL-5 expression), there was an inverse correlation between IL-5 and IFN- $\gamma$  ( $-0.607$ ,  $P < .05$ ).

IL-10 protein data did not show any differences between groups. Among Belgian patients, 14 of 26 were asthmatic, whereas only 2 of the 29 south Chinese patients were asthmatic ( $P < .0001$ ). In asthmatic versus non-asthmatic Be-NPs, TGF- $\beta$ 1 levels were significantly increased; no further changes caused by asthma comorbidity were found in this group (Table II). We therefore show the TGF- $\beta$ 1 data of the polyps from nonasthmatic patients, whereas other data are demonstrated with all patients included.

### DISCUSSION

Here we show, for the first time, that although clinical appearance, mucosal edema formation, T-effector cell activation and regulatory T-cell impairment (specifically the downregulation of Foxp3, a signal transduction factor related to regulatory T

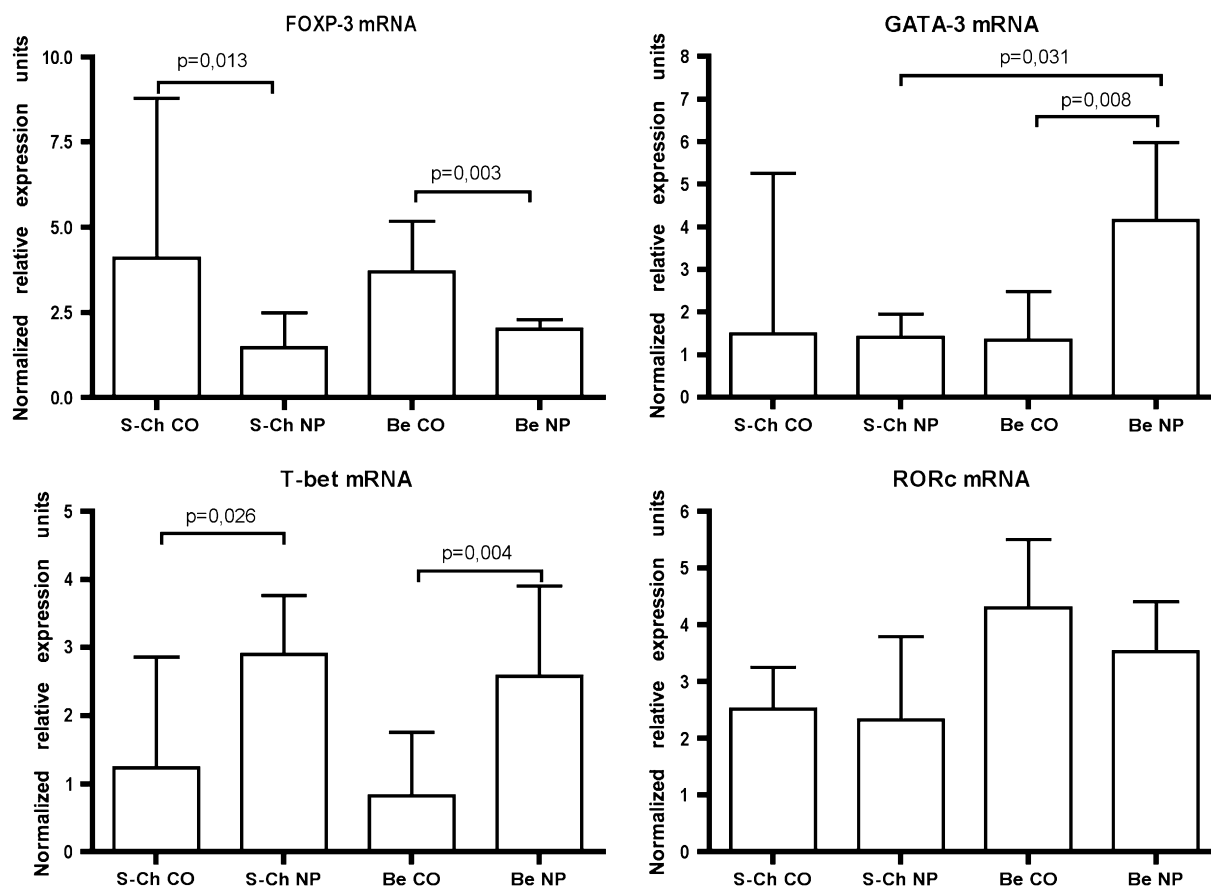


**FIG 1.** Semiquantitative evaluation of granulocyte tissue infiltration. Values are reported as medians and interquartile ranges for each group. The significance level was set at an  $\alpha$  value of .05. Concentrations for ECP and MPO are reported as medians and interquartile ranges for each group, and the significance level equals a *P* value of .05. From these data, an ECP/MPO ratio was calculated. HE, Hematoxylin.

cells) are shared by polyps from European and Asian patients, the inflammation pattern is remarkably different between the disease groups, with a  $T_H1/T_H17$  dominance in south Chinese patients and a  $T_H2$  dominance in Belgian patients. These differences are reflected by a distinguished neutrophil and eosinophil granulocyte activation bias in polyps from Asian and European patients, respectively.

Nasal polyposis, also referred to as CRSwNP, in European and US white subjects represents an often severe and difficult-to-treat eosinophilic airway inflammation, which frequently is linked to

comorbid asthma.<sup>1,2</sup> The typical inflammation shares many features with asthma, such as increased numbers of mucosal eosinophils; activated T cells producing a  $T_H2$ -biased cytokine profile, including IL-5 and eotaxin; and IgE formation.<sup>17</sup> Clinically, these airway diseases show a late onset, patients often are dependent on corticosteroid medication, and patients might have aspirin-exacerbated respiratory disease. In the current understanding of CRSwNP, the characteristic eosinophilic inflammation is linked to extracellular matrix destruction and edema formation, possibly mediated by toxic eosinophil-derived products.<sup>18</sup> However, the



**FIG 2.** mRNA levels for T-bet, GATA-3, RORc, and Foxp3 transcription factors normalized to the levels of  $\beta$ -actin, hydroxymethylbilane synthase, and elongation factor 1 as housekeeping genes in each group. Values are reported as medians and interquartile ranges for each group. The significance level was set at a *P* value of .05.

exact pathomechanism of the inflammation–remodeling link has not been fully elucidated.

Of note, clinically comparable upper airway disease also seems to exist without abundantly activated tissue eosinophils.<sup>12</sup> Here we extend our observations and demonstrate that NPs from south Chinese patients, which show a neutrophilic cellular pattern similar to those of other Asian regions, such as Thailand,<sup>13</sup> and NPs from Belgian patients do not share the same T-effector cell polarization. S-Ch NPs display clear  $T_H1$  and  $T_H17$  signals, including T-bet expression and IFN- $\gamma$  protein formation, and IL-17 and the related cytokines IL-1 $\beta$  and IL-6 protein synthesis in tissue homogenates, whereas Be NPs demonstrate an upregulated GATA-3 transcription factor in line with increased IL-5 protein levels compared with those seen in control tissue. Additionally, the eosinophil-specific CC chemokine eotaxin, ECP as a readout of severity of local eosinophil activation, and total IgE as another  $T_H2$  stigma are significantly upregulated versus levels seen in control tissue and S-Ch NPs, confirming former reports.<sup>5</sup>

To establish the dominance of specific granulocytes in mucosal inflammation, we counted cell numbers and measured markers of activation, such as ECP for eosinophils and MPO for neutrophils. First, the number of eosinophils was significantly lower in S-Ch NPs compared with levels in Be NPs, and second, the eosinophils in S-Ch NPs did not show an increased activation in terms of ECP release versus that seen in respective control tissue. It appeared that there was a dissociation between the presences of eosinophils

and their activation status in south Chinese patients. In contrast, neutrophil numbers were significantly increased versus those seen in control subjects in both Be NPs and S-Ch NPs, and MPO concentrations were increased in both NP groups, although they only reached significance for Be NPs. However, in total, this resulted in an ECP/MPO ratio that demonstrated a granulocyte activation bias in favor of eosinophils for Be NPs and neutrophils in S-Ch NPs. The mechanisms orchestrating the granulocytes in the different patient populations do require further elucidation.

T-bet and GATA-3 are regulators of  $T_H1/T_H2$  differentiation that specifically function in  $T_H$  cells. T-bet, a member of the T-box family of transcription factors, is strongly correlated with IFN- $\gamma$  expression and is specifically upregulated in primary  $T_H$  cells that differentiate along the  $T_H1$  pathway. T-cells from T-bet<sup>-/-</sup> mice show defective IFN- $\gamma$  production, and retroviral expression of T-bet has been described to suppress expression of the  $T_H2$ -type cytokines IL-4 and IL-5.<sup>17</sup> On the other hand, GATA-3 is a key regulator of  $T_H2$  cytokine production, and its expression is enhanced in polarized  $T_H2$  cells and downregulated in  $T_H1$  cells. Moreover, forced expression of GATA-3 in polarized  $T_H1$  cells is sufficient to initiate  $T_H2$  cytokine expression.<sup>18</sup> GATA-3 is thought to play an important role in the expression of  $T_H2$  cytokines in asthma. Enhanced expression of GATA-3 has been observed in the airways of asthmatic patients, with a further increase on segmental allergen challenge.<sup>19,20</sup> We do show here that the transcription factor expression is translated in appropriate

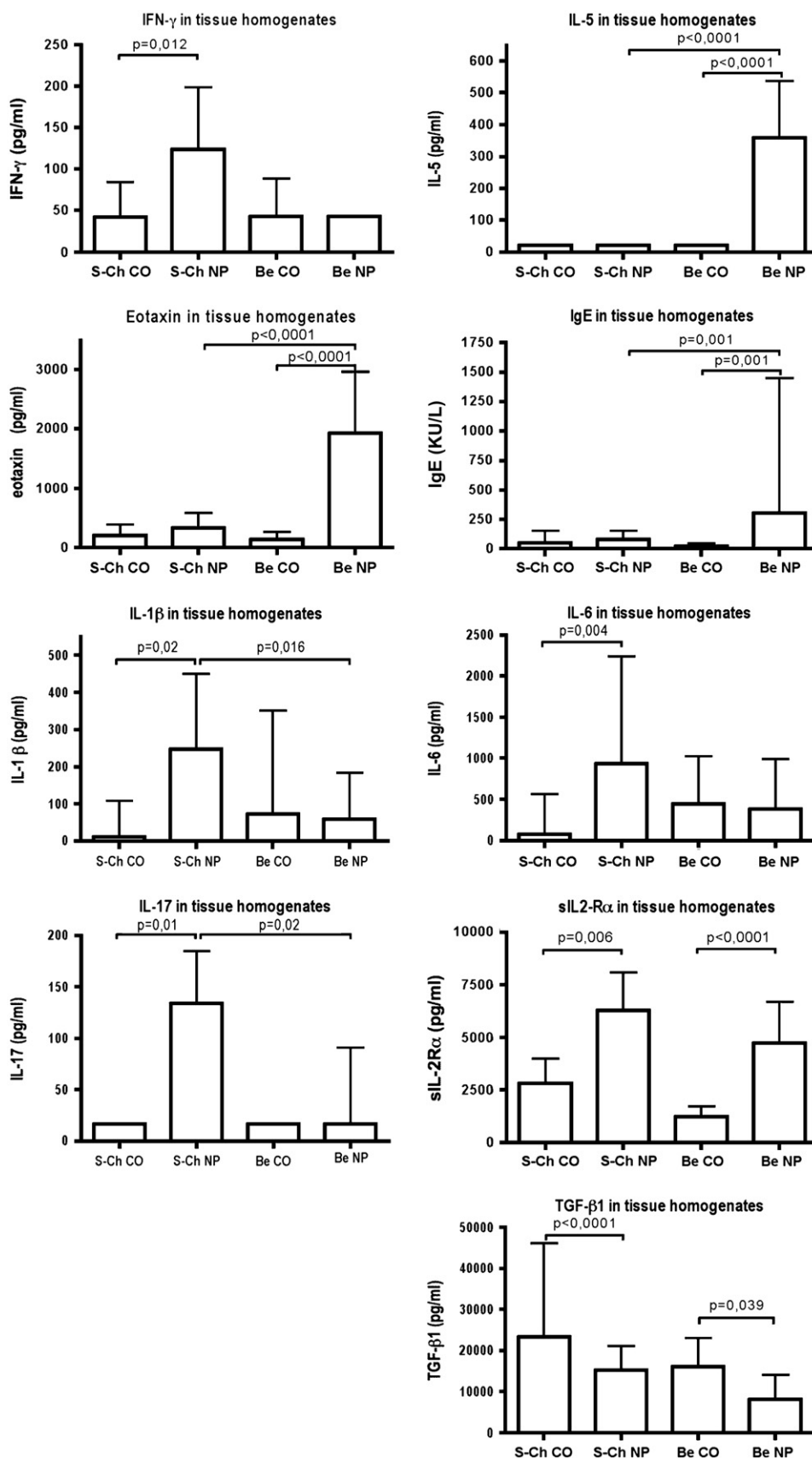


FIG 3. (See next page for legend).



**TABLE II.** Cytokine concentrations and cell numbers in the Belgian CRSwNP subgroups with and without asthma comorbidity

	CRSwNP with asthma (n = 14)	CRSwNP without asthma (n = 12)	P value
Edema score (semiquantitative)	1.0 (1.0)	1.0 (1.0)	.6
Eosinophils (hematoxylin stain), n	21.0 (17.0)	14.0 (10.0)	.145
Neutrophils (MPO), n	20.0 (10.0)	18.0 (3.0)	.373
ECP ( $\mu\text{g/L}$ )	19,470 (18,920)	15,675.0 (28,468.0)	.797
MPO (ng/mL)	9588.4 (20,429.6)	10,230.9 (15,709.4)	1.000
sIL-2R $\alpha$ (pg/mL)	3787.1 (10025.3)	5357.8 (5099.8)	.898
IL-5 (pg/mL)	382.7 (394.9)	228.8 (1432.3)	.606
Eotaxin (pg/mL)	2585.3 (4495.6)	11,874.3 (2974.9)	.606
IFN- $\gamma$ (pg/mL)	42.9 (0.0)	42.9 (189.7)	.518
IgE (KU/L)	880.0 (2454.6)	190.3 (3088.2)	.518
IL-1 $\beta$ (pg/mL)	40.2 (74.1)	59.1 (960.3)	.192
IL-6 (pg/mL)	930.3 (2204.3)	346.6 (2253.7)	.213
IL-17 (pg/mL)	41.50 (51.30)	16.6 (290.1)	.730
TGF- $\beta$ (pg/mL)	10,765.8 (25,653.9)	7745.6 (26,501.5)	.0233

Data are expressed as medians and interquartile ranges. A significant difference was only noted for TGF- $\beta$ 1 protein.

sIL-2R $\alpha$ , Soluble IL-2 receptor  $\alpha$ .

T<sub>H</sub>1/T<sub>H</sub>2 cytokine protein expression; however, there seems to be a posttranslational suppression of IFN- $\gamma$  expression in Be NPs, which requires further clarification. Of interest, there was an inverse correlation between IL-5 and IFN- $\gamma$  in Be NPs.

In mice T<sub>H</sub>17 cells have been established as a separate lineage of T<sub>H</sub> cells distinct from conventional T<sub>H</sub>1 and T<sub>H</sub>2 cells, and information in human subjects is becoming available. Transcription factors and signaling molecules that are important for the differentiation of T<sub>H</sub>1 or T<sub>H</sub>2 cells, including signal transducer and activator of transcription (STAT) 1, STAT4, STAT6, and T-bet, are dispensable for the development of T<sub>H</sub>17 cells.<sup>21,22</sup> Recently, ROR $\gamma$ t (equivalent to RORC in human subjects) was discovered as a novel T<sub>H</sub>17 transcription factor.<sup>23</sup> T<sub>H</sub>17 cells, through IL-17, induce the production of proinflammatory mediators, such as IL-1 and TNF- $\alpha$ , induce receptor activator of nuclear factor  $\kappa$ B ligand expression, stimulate transcriptional nuclear factor  $\kappa$ B activity, and induce IL-6 and IL-8 secretion in fibroblasts, endothelial cells, and epithelial cells.<sup>24,25</sup> Furthermore, *in vitro* studies showed the induction of metalloproteinases by IL-17, which can lead to the characteristic edema formation in NPs of patients with cystic fibrosis, characterized by abundant neutrophils and increased concentrations of IL-8 and MPO.<sup>5</sup> In this article we demonstrate increased concentrations of several cytokines related to T<sub>H</sub>17 cells, such as IL-1 $\beta$ , IL-6, and IL-17A, in S-Ch NPs, supporting the effect of this T cell in this specific group of patients, although an upregulation of RORC was not demonstrated here. Thus T<sub>H</sub>17 cells, rather than T<sub>H</sub>2 cells, might play a critical role in chronic neutrophilic inflammatory upper airway disease in Asian compared with white patients.

The findings of a downregulation of Foxp3 expression in NPs versus control tissues, both for Be NPs and S-Ch NPs, is in line with a recent article in which we supported these findings obtained by means of immunohistochemistry for Foxp3.<sup>14</sup> Foxp3 was initially thought to be a specific marker for natural regulatory T cells and could not be activated in peripheral T cells<sup>26-28</sup>; however,

recent studies demonstrated the induction of Foxp3 in induced regulatory T cells as well.<sup>29-31</sup> Regulatory T cells play an important role in controlling T<sub>H</sub>2 immune responses, and an impaired expansion of natural, inducible, or both types of regulatory T cells has been suspected to result in the development of allergy and asthma.<sup>32</sup> A defective suppressive function of regulatory T cells in patients with CRSwNP, indicated here by the low Foxp3 expression, might account for the persistent inflammation in polyps from white and Asian patients, although the underlying mechanisms might be different. It has been suggested that IL-4 present at the time of T-cell priming inhibits Foxp3 expression by means of direct binding of GATA-3 to the Foxp3 promoter.<sup>33</sup>

Clinical symptoms (nasal obstruction and loss of smell) and results of nasal endoscopy (typical appearance of polyps), sinus CT scanning, and histology were perfectly comparable in the 2 polyp groups, both qualitatively and quantitatively. The only exception from this was asthma comorbidity, which was significantly more frequent in Be NPs versus S-Ch NPs; whether this difference is related to the involvement of activated eosinophils, which are recruited from the bone marrow and might circulate to other target organs, such as the lungs,<sup>34</sup> in Be NPs but not in S-Ch NPs is currently unclear.

We had to correct for asthma comorbidity when comparing the regulation of TGF- $\beta$ 1 in Be NPs because asthmatic subjects showed a significant upregulation of TGF- $\beta$ 1 protein expression versus NPs from nonasthmatic patients. However, because asthma comorbidity was rare in the S-Ch NPs, it seems legitimate to compare nonasthmatic subgroups with NPs, and both groups demonstrated a significant downregulation of TGF- $\beta$ 1 in the tissue. TGF- $\beta$ 1 is a profibrotic factor, increases the deposition of extracellular matrix proteins, and impairs the balance between metalloproteinases and their natural inhibitors to limit ECM degradation<sup>21</sup>; however, in patients with CRSwNP, in clear contrast to patients with chronic rhinosinusitis without NPs,<sup>5</sup> those features are lacking, which is in line with the relative deficit in

**FIG 3.** Concentrations of soluble IL-2 receptor  $\alpha$  (sIL-2R $\alpha$ ), IL-5, IFN- $\gamma$ , IL-1 $\beta$ , IL-6, IL-17A, eotaxin, IgE, and TGF- $\beta$ 1 protein in each group. Values are reported as medians and interquartile ranges for each group. The significance level was set at a P value of .05. Please note that the TGF- $\beta$ 1 protein data refer to patients without asthma (see the Results section).

TGF- $\beta$ 1 production described here and before.<sup>14</sup> We cannot fully answer the question of whether the increased TGF- $\beta$ 1 protein expression in NPs of asthmatic patients reflects a more severe eosinophil involvement, as has been described before,<sup>22</sup> or distinguishable disease subgroups.

In summary, our findings suggest that treatment approaches to upper airway disease, such as nasal polyposis, might have to be different in different environments. Thus far, in daily practice in Europe and the United States, we are focused on the treatment of eosinophils as a major target, use topical and systemic corticosteroids as first-line therapy for NPs, and have also used humanized anti-IL-5 mAbs in recent studies.<sup>35</sup> However, such concepts will most probably be inappropriate in Asian patients with neutrophilic IL-17–biased polyps. A new view on the type and role of inflammation in airway disease needs to be elaborated, which might lead to distinct therapeutic approaches and outcome parameters for specific types of inflammation.

**Clinical implications: The different T effector cell polarizations are likely to influence the choice of therapeutic approaches to this disease.**

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