ORIGINAL ARTICLE EXPERIMENTAL ALLERGY AND IMMUNOLOGY

The association between bacterial colonization and inflammatory pattern in Chinese chronic rhinosinusitis patients with nasal polyps

L. Ba[1,2*], N. Zhang[3*], J. Meng[1], J. Zhang[4], P. Lin[4], P. Zhou[1], S. Liu[1] & C. Bachert[3]

[1]Department of Otorhinolaryngology, Head and Neck Surgery of West China Hospital, Chengdu, Sichuan; [2]Department of Otorhinolaryngology of people's Hospital of Tibet autonomous region, China; [3]The Upper Airways Research Laboratory, Department of Otorhinolaryngology, Ghent University, Ghent, Belgium; [4]The Upper Airway Research Laboratory, West China Hospital, Sichuan University, Chengdu, China

To To cite this article: Ba L, Zhang N, Meng J, Zhang J, Lin P, Zhou P, Liu S, Bachert C. The association between bacterial colonization and inflammatory pattern in Chinese chronic rhinosinusitis patients with nasal polyps. Allergy 2011; DOI: 10.1111/j.1398-9995.2011.02637.x

Keywords
bacterial colonization; eosinophils; nasal polyps; neutrophils; T-cell subsets.

Correspondence
Professor Shixi Liu, PhD, MD, Department of Otorhinolaryngology, Head & Neck Surgery, West China Hospital, Sichuan University, Sichuan, Chengdu 610041, 2010-09-22 China.
Tel.: (86) 028 81812403
Fax: (86) 028 85422439
E-mail: liusx999@163.com

Accepted for publication 15 April 2011

DOI:10.1111/j.1398-9995.2011.02637.x

*These authors contributed equally to this work.

Edited by: Marek Sanak

Abstract

Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) can be subdivided according to the mucosal inflammatory patterns. In mainland China, apart from interleukin (IL)-5-positive and IL-17-positive polyps, a large group of patients with IL-5/IL-17/interferon-gamma (IFNγ)-negative nasal polyps (referred to as key cytokine-negative (KCN) polyps) can be found.
Objective: To further study the KCN polyps and evaluate the associations between bacterial colonization and mucosal inflammatory pattern in KCN *vs* IL-5-positive nasal polyps.
Methods: Nasal polyp or nasal turbinate tissue was obtained from 89 Chinese CRSwNP patients and 36 nonatopic control subjects during surgery. Samples without and after SEB exposure were processed for the assessment of pro-inflammatory cytokines and mediators by immunoassay. Prior to surgery, nasal swabs were taken from each patient for microbiological evaluation.
Results: Overall, 80% polyp tissue did not express IL-5, with about 70% (49/71) of these being KCN. Key cytokine-negative nasal polyps were characterized by the synthesis of mediators promoting neutrophilic inflammation (myeloperoxidase (MPO), IL-1β, IL-6 and IL-8), whereas IL-5-positive nasal polyps were characterized by the synthesis of mediators promoting eosinophilic inflammation (IL-5, ECP, total IgE and SE-IgE). Key cytokine-negative nasal polyps were associated with greater Gram-negative bacterial load compared with controls, while IL-5-positive nasal polyps were associated with greater Gram-positive bacterial colonization *vs* controls and KCN polyps.
Conclusion: Our findings suggest that the bacteria colonizing nasal polyps of CRSwNP patients may impact on or be determined by the presence/absence of IL-5.

Abbreviations:
CRS, chronic rhinosinusitis; CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; ECP, eosinophil cationic protein; IgE, immunoglobulin E; IFNγ, interferon gamma; IL, interleukin; KCN, key cytokine negative; MPO, myeloperoxidase; SE (A/B/C), *Staphylococcus aureus* enterotoxin (A/B/C); sIL, soluble interleukin; TIM, T-cell immunoglobulin mucin domain; TGF-β1, transforming growth factor-β1; T (H), T helper; TLRs, Toll-like receptors.

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nasal and paranasal mucosa (1), which has been increasing in prevalence and incidence worldwide and affects between 4% and 28% of the population in the western countries (2, 3). Pathophysiological studies have indicated that CRS can be classified into two subgroups based on the absence (CRSsNP) or presence (CRSwNP) of nasal polyps, which have distinctive inflammatory cell and mediator profiles (4–7) and specific features of re-modelling (8, 9). Earlier

studies in Caucasian patients demonstrated that the CRSwNP subgroup was characterized by a T-helper (TH)2-skewed eosinophilic inflammation comprising significantly higher levels of interleukin (IL)-5, IL-13, eotaxin, and eosinophil cationic protein (ECP), whereas the CRSsNP subgroup was characterized by a TH1 inflammatory response comprising significantly higher levels of interferon gamma (IFNγ) and transforming growth factor beta (TGFβ).

Recently, we have compared the remodelling and inflammatory patterns in the upper airways of Caucasian and Chinese CRSwNP patients and demonstrated that there are clear differences in the inflammatory patterns in these ethnicity groups (10–12). As nasal polyps are often linked to comorbid asthma and a difference has also been shown in the prevalence of comorbid asthma in Asian and Caucasian patients (7), more recently, we evaluated the asthma-associated mucosal factors in nasal polyps from a cohort of 70 Belgian and 93 Chinese CRSwNP patients, of whom 34% and 8% patients, respectively, had comorbid asthma (13). Our study demonstrated that the risk for comorbid asthma in both the Belgian and Chinese patients was significantly associated with expression of IL-5, presence of Immunoglobulin E (IgE) antibodies to *Staphylococcus aureus* enterotoxins (SEs) and total IgE in the nasal polyps of these individuals. Moreover, there were marked differences in the expression of some key cytokines (IL-5, IL-17 and IFNγ) in the nasal polyps of the two patient groups. In particular, 28% of nasal polyps from Chinese patients were unable to express these key cytokines (i.e. were 'key cytokine-negative' (KCN) nasal polyps), compared with only 4% of nasal polyps from Caucasian patients. In view of the potentially pivotal role of SEs in mediating inflammation and key cytokines in the nasal polyps of CRSwNP patients, the aim of this study was to evaluate any associations between bacterial colonization and mucosal inflammatory pattern in CRSwNP patients, specifically in polyps with the cytokine patterns found most frequently in mainland China, i.e. IL-5-positive and KCN (IL-5, IL-17 and IFNγ) nasal polyps.

Materials and methods

Nasal polyp/turbinate tissue

Nasal polyp tissue was obtained from 89 CRSwNP patients during routine endonasal sinus surgery, at the Department of Oto-Rhino-Laryngology, West China Hospital, and all samples were either snap-frozen in liquid nitrogen on collection and stored at −80°C until further assessment by immunoassay or used as fresh tissue whenever available.

The diagnosis of sinus disease in each patient was based on history, clinical examination, nasal endoscopy and computed tomography (CT) scan of the sinuses according to the EP3OS guidelines (14). The incidence and severity of individual nasal symptoms were graded according to a scale of 0–3 (no symptoms, mild symptoms, moderate symptoms and severe symptoms), and the atopic status of the patient was confirmed by the Phadiotop test (Phadia, Uppsala, Sweden). The diagnosis of asthma was made by a pneumologist. None of the subjects had used oral or nasal corticosteroids or antibiotics more than 3 weeks before surgery. Similarly, nasal turbinate tissue was also collected from 36 healthy nonatopic control subjects, presenting for septoplasty because of anatomical variations at the Department of Oto-Rhino-Laryngology.

All patients gave written informed consent for collection and use of surgically obtained tissue, and the study was approved by the local ethics committee of the West China Hospital of Sichuan University, Chengdu, China.

Assessment of cytokines and IgE levels in tissue homogenates

Snap-frozen nasal polyp and turbinate tissue specimens were processed and assayed for cytokines and SE or total IgE levels as previously described in detail (10–12).

Assessment of SEB-induced synthesis of cytokines from cultured IL-5-positive and KCN nasal polyp tissue

Freshly obtained nasal polyp samples from nine IL-5-positive and eleven KCN subsets of CRSwNP patients were washed and suspended in 10 ml of sterile RPMI 1640 tissue culture medium (Sigma–Aldrich, Bornem, Belgium), supplemented with 2 mM L-glutamine (Invitrogen, Merelbeke, Belgium), antibiotics (50 IU/ml penicillin and 50 mg/ml streptomycin) (Invitrogen) and 0.1% bovine serum albumin (BSA; Sigma, Flanders, NJ, USA). The tissue was cut into smaller pieces and passed through a fine wire mesh (pore size 0.9 mm^3) to obtain tissue fragments. Following three washings with fresh culture medium, the tissue fragments were resuspended at 0.04 g tissue/ml culture medium. A 0.5-ml aliquot of each suspension was dispensed into a separate well of a 48-well tissue culture plate (BD Falcon; VWR, Leuven, Belgium) and incubated in the absence (negative control) or presence of 0.5 μg/ml SEB (Sigma-Aldrich, Bornem, Belgium) for 24 h, as described previously (15). Culture medium was collected from each well and stored at −20°C until analysis for cytokines IL-1β, IFNγ, IL-2, IL-5, IL-8, IL-6 and IL-17, using commercially available Quantikine ELISA kits (R&D Systems, Minneapolis, MN, USA).

The subsets for SEB exposure have been selected at random from the respective groups, and baseline cytokine expression showed no difference between stimulated and non-stimulated samples.

Assessment of bacterial colonization

Immediately prior to surgery, samples were obtained by swabbing the middle nasal meatus of both sides and inoculated sterile to sheep blood agar (DiMed, St. Paul, MN, USA), eosin methylene blue agar (Baltimore Biological Laboratories, Cockeysville, MD, USA) and chocolate agar (Bio-Merieux, Marcy l'Etoile, France). All cultures were incubated at 25–37°C under aerobic or anaerobic conditions as required. The cultures were observed at 24, 48 or 72 h, and individual bacterial strains were identified according to standard bacteriological methods (16). Samples were considered

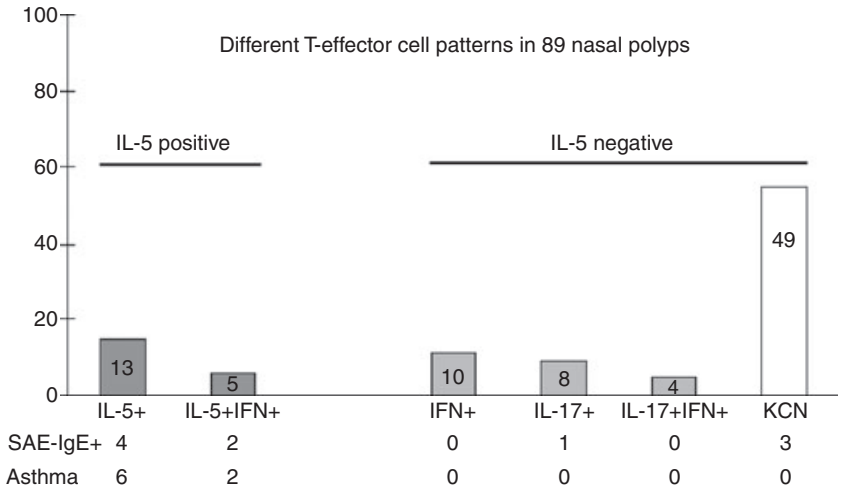

Figure 1 Differentiation of nasal polyps from Chinese chronic rhinosinusitis with nasal polyps patients, based on key T-effector cell cytokine (IL-5, IL-17 and IFNγ) expression. Furthermore, the presence of SAE-immunoglobulin E in serum and asthma is noted.

to be negative for bacterial colonization when no growth was detected after 72 h. After 3 days of culture, a turbidometrically controlled suspension of individual pure bacterial colonies was transferred to three Vitek identification cards [GP for all Gram-positive cocci (identifying 45 taxa), GN for all Gram-negative bacilli (116 taxa) and YST for all yeast-like organisms (36 taxa) (bioMérieux, Shanghai, China)] according to the manufacturer's instructions: The cards contain 29 different biochemical broths in reaction cells and one negative control cell to assess the growth and viability of the suspension. Incubation times vary from 2 to 48 h depending on the growth rate of the organisms. The automated microbe identification analyser (VITEK 2 compact; bioMérieux, Marcy l'Etoile, France) was used to determine growth by measuring light attenuation with an optical scanner. Data obtained with the ID 32E system were read automatically and interpreted with the database version 3.0.

Statistical analysis

Statistical analyses were performed using the SPSS version 12.0 software (SPSS Inc, Chicago, IL, USA). Data were expressed as median and interquartile ranges (IQR). Baseline variables were analysed using a one-way ANOVA test and the Fisher's exact text or likelihood ratio. The Kruskal–Wallis test was used to assess the significance of intergroup variability using paired comparisons, and the Mann–Whitney *U* 2-tailed test was used to assess significance for between-group comparisons. *P* values ≤0.05 were considered to be statistically significant.

Results

Patient characteristics and cytokine patterns in the nasal polyps of Chinese CRSwNP patients

Figure 1 shows the number of samples from CRSwNP patients spontaneously synthesizing the key cytokines IL-5, IL-17 and IFNγ. Overall, 80% of the nasal polyps were IL-5 negative, with 55% (49/89) synthesizing none of these key cytokines (i.e. KCN nasal polyps). Detection limits are provided in Table 1.

Table 1 Detection limits of the assays used for this study

Key cytokine (pg/ml)	IL-5	IFN-g	IL-17	L-22	IL-9
Detection limit	0.6	7.8	0.6	3.9	1.9
Detection limit(after sample /handling dilution)	7.0	42.9	7.0	42.9	20.9

Retrospective assessment of the patient characteristics based on their ability to synthesize IL-5 and the key cytokines indicated that the patients with IL-5-positive and KCN polyps were not significantly different with regard to demographic or clinical characteristics, apart from the number of patients with asthma comorbidity in the IL-5-positive group (33% *vs* 0% in KCN group; $P < 0.05$). In contrast, both the IL-5-positive and KCN groups were significantly different from the control group with regard to their clinical characteristics, as expected (Table 2).

Comparison of inflammatory mediators, IgE and cytokines in IL5-positive and KCN NPs

The presence of IL-5 protein in nasal polyp tissue was associated with significantly increased concentrations of ECP, total IgE and SE-IgE, compared with nasal polyp tissue from KCN subjects (Fig. 2). Similarly, IL-5-positive nasal polyps also demonstrated significantly increased amounts of IL2sR-alpha, IL-8 and myeloperoxidase (MPO), compared with nasal tissue from control subjects. In contrast, in KCN nasal polyps, MPO, IL-1b, IL-2sRa, IL-6, IL-8 and TGFβ1 were significantly increased compared with nasal tissue from

Table 2 Patient characteristics and symptom scores in the nasal tissue of control subjects and patients with IL-5-positive and key cytokine-negative nasal polyps (NP)

Parameter	Control	IL-5-positive. NP	Key cytokine-negative. NP	ANOVA/likelihood ratio*		
No of patient	36	18	49			
Age (years/mean; range)	29 (18–53)	48 (34–60)	38 (19–56)		NS	
Women/men	13/23	11/7	19/30		NS*	
Asthma in history	0/0	8/18	0/49	∮0.001*	∫NS*	♂0.023*
Positive Phadiotop	0/36	5/18	8/49	∮0.023*	∫NS*	♂NS*
Disease duration (year)	0 (0–0)	7.50 (1–20)	8.5 (0.5–28)	NS		
CT score (Lund)	0 (0–0)	14.4 (10–23)	14.7 (0–23)	∮0.0001	∫0.0001	♂NS
Polyp score (Davos)	0 (0–0)	4.6 (4–6)	4.17 (2–6)	∮0.0001	0.0001	♂NS
Symptom Visual analogue scales (VAS) scores						
Loss of smell	0 (0–0)	2 (0–3)	1 (0–3)	∮0.0001	∫0.0001	♂NS
Postnasal drip	0 (0–0)	1 (0–2)	1 (0–2)	∮0.0001	∫0.0001	♂NS
Headache	0 (0–1)	1 (0–2)	1 (0–3)	∮0.034	∫0.045	♂NS
Feel troubled	0 (0–0)	2 (2, 3)	2 (0–3)	∮0.0001	∫0.0001	♂NS
Blocked nose	0 (0–1)	2 (0–3)	2 (0–3)	∮0.001	∫0.001	♂NS
Sneezing	0 (0–0)	1 (0–3)	1 (0–3)	∮0.041	∫NS	♂NS
Runny nose	0 (0–0)	2(0–3)	2 (0–3)	∮0.0001	∫0.0001	♂NS
Itchy nose	0 (0–0)	1 (0–3)	0 (0–2)	∮0.001	∫0.01	♂0.047

Data are expressed as medians and interquartile ranges. The level of significance (*P*) was obtained by means of ANOVA or likelihood ratio (marked with *). *P*-values of ≤0.05 was considered statistically significant. For comparisons of continuous variables between two groups, the Mann–Whitney *U* test was performed. (NS, not significant; ∫, control *vs* KCN NPs; ∮, control *vs* IL-5 + NP; ♂, IL-5 + NP *vs* KCN NPs).

control subjects. Indeed, assessment of the mean ECP/MPO ratio indicated an eosinophilic preponderance for IL-5-positive nasal polyps (3.16 ± 5.04) and a neutrophilic preponderance for KCN nasal polyps (0.37 ± 0.98). Assessment of IL-9 and IL-22, however, indicated that these cytokines were not synthesized to a significant amount by either polyp subset.

SEB-induced inflammatory mediator release from cultured IL-5-positive and KCN nasal polyp subsets

After 24 h in culture, the spontaneous release of IL-8 was significantly higher from KCN nasal polyps compared with IL-5-positive nasal polyps ($P = 0.01$), whereas spontaneous IL-5 release was only found in IL-5-positive nasal polyps (Fig. 3). In contrast, spontaneous release of IL-1β, IL-2, IL-6, IL-17 and IFNγ was not significantly different between IL-5-positive and KCN nasal polyps.

Stimulation with SEB led to significantly greater release of IFNγ and IL-2 from KCN nasal polyps after 24 h than from IL-5-positive nasal polyps ($P = 0.04$ and $P = 0.02$, respectively). As expected, SEB-induced IL-5 release was significantly higher from IL-5-positive nasal polyps than from KCN nasal polyps ($P < 0.01$).

Microbiologic patterns in IL-5-positive and KCN polyp subsets

Microbiologic assessment of the nasal passages of CRSwNP patients and controls demonstrated that the numbers of colonizing microorganisms were significantly increased in both IL-5-positive and KCN nasal polyp patient groups compared with controls ($P = 0.004$ and $P = 0.003$, respectively) (Table 3). Furthermore, significantly more IL-5-positive nasal polyp patients carried Gram-positive bacteria than KCN nasal polyp patients or controls ($P = 0.001$ for both). In contrast, Gram-negative bacteria were isolated more frequently in KCN nasal polyp patients than in IL-5-positive nasal polyp patients and control subjects, reaching statistical significance for the latter.

Discussion

Our study in mainland China confirmed that among CRS patients with nasal polyps, a substantial proportion (80%) did not express detectable IL-5 in the nasal polyp tissues. Moreover, nearly 70% (49/71) of these IL-5-negative nasal polyps were spontaneously not expressing detectable levels of IL-17 or IFNγ, which we describe as KCN nasal polyps. Furthermore, these polyps also did not demonstrate relevant concentrations of IL-9 or IL-22, which may account for other T-helper cell populations. Assessment of the inflammatory mediators present in the two subsets of nasal polyps demonstrated that MPO, IL-1b, IL-6 and IL-8 were significantly increased in the KCN nasal polyps, with a mean ECP/MPO ratio of 0.37 suggesting predominance for neutrophilic inflammation. In contrast, concentrations of ECP, total IgE, and SE-IgE, and IL-5 were significantly increased in the IL-5-positive nasal polyps compared with KCN nasal polyps, with predominance for eosinophilic inflammation suggested by a mean ECP/MPO ratio of 3.16. Interestingly, our study showed that the KCN nasal polyps could be induced to release IL-2 and IFNγ by stimulation with SEB, thus empha-

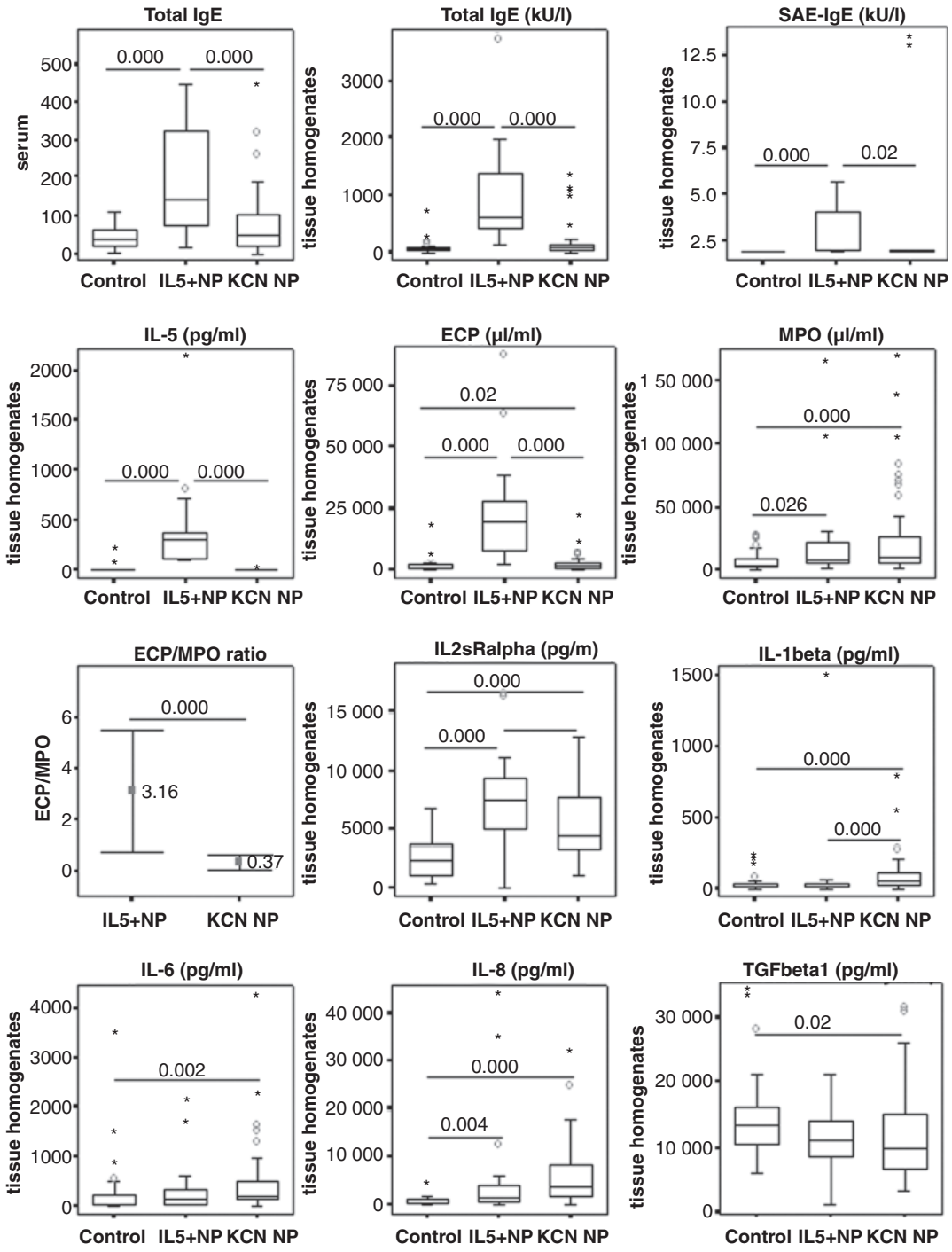

Figure 2 Expression of inflammatory cytokines, mediators, total and SAE-Immunoglobulin E (IgE) in IL-5-positive and key cytokine-negative (KCN) nasal polyp tissue from Chinese chronic rhinosinusitis with nasal polyps patients and nasal turbinate tissue from control subjects (the Mann–Whitney *U* 2-tailed test was used for between-group comparison, with significance level set at $P \leq 0.05$). The IL-5 + polyps are characterized by an increase in total and *Staphylococcus aureus*-specific IgE, whereas the KCN polyps are myeloperoxidase, IL-1, IL-6 and IL-8 positive. IL-2R alpha is increased in both groups over placebo, indicating the activation of an unknown T-cell population in the KCN polyps.

sizing a Th1-biased neutrophilic inflammatory profile, whereas IL-5-positive polyps responded with the release of IL-5. Moreover, retrospective analysis of the association between the cytokine profiles of the nasal polyp subsets and the clinical characteristics of patients indicated that not a single patient with KCN nasal polyps showed any history of

Table 3 Microbial species isolated from the nasal passages of control subjects and patients with IL-5-positive and key cytokine-negative nasal polyps (NPs)

Microorganism	Key cytokine-negative NP *n* = 49	IL-5-positive NP *n* = 18	Control *n* = 36	Chi-squared test (Fisher's exact *)
Aerobe & facultative anaerobe				
Gram-positive bacteria				
Corynebacterium	3	1	1	
B. subtilis	2	1	1	
Coagulase-negative staphylococci	3 (6%)	3 (17%)	1 (3%)	NS*
Staphylococcus aureus	1 (2%)	2 (11%)	0	0.24*
Staphylococcus epidermidis	1 (2%)	4 (22%)	2 (3%)	NS*
Streptococcus pneumonia	2	2		
Streptococcus viridians	2		2	
Propioni Streptococcus	1		1	
G^+ Total microorganism	15 (31%)	13 (72%)	8 (22%)	∫NS ∮0.001 ♂0.001
Gram-negative bacteria				
Escherichia coli	3			
Citrobacter freundii	1			
Proteus mirabilis	1			
Enterobactor cloaca	2		1	
Enterobacter aerogenes	2			
Morganella morganii	2	1		
Klebsiella pneumonia	1	1	2	
Haemophilus influenza	2	1	2	
Neisseria	2			
G^- Total microorganism	17 (35%)	3 (17%)	5 (14%)	∫0.05 ∮NS ♂ NS
Anaerobe bacteria				
Propionibacterium species	1			
Fungi				
Yeast-like fungus	1		2	
Candida albicans	1			
Multimicrobial grow	3		4	
No bacterial grow	11 (23%)	2 (11%)	17(48%)	∫0.003 ∮0.004 ♂ NS
Total samples with microorganisms	38 (77%)	16 (89%)	19 (53%)	∫ 0.004 ∮0.003 ♂ NS

The number of patients with grow of any microorganisms was significantly higher in both NP groups and control. Significantly more nasal polyps carried Gram-positive bacteria in the IL-5 + NP than in the other groups. In contrast, Gram-negative bacteria were significantly more frequent in KCN NPs than in controls. Statistical analysis was performed using the chi-square test or Fisher's exact test (marked with *). Significance was accepted for $P \leq 0.05$. (NS, not significant; ∫; control *vs* KCN NPs; ∮, control *vs* IL-5 + NP; ♂, IL-5 + NP *vs* KCN NPs).

comorbid asthma, whereas nearly half the patients with the IL-5-positive nasal polyps were asthmatics.

We here describe for the first time that evaluation of the associations between the nasal polyp subsets and nasal bacterial colonization indicated more KCN nasal polyp patients were found to carry Gram-negative bacteria in the nasal passages compared with IL-5-positive nasal polyp patients, while significantly more IL-5-positive nasal polyp patients were found to carry Gram-positive bacteria than KCN nasal polyp patients. However, the colonization rate of polyps and also control subjects with *S. aureus* in the Chengdu population compared to what has been reported from Europe appears low. Supporting our findings, it has been found recently that only 6.5% of intensive care unit (ICU) physicians and only 1.99% of the non-ICU physicians were nasal *S. aureus* carriers in the largest hospital of Chengdu, China (17).

Collectively, our findings confirm the findings of a dichotomy of Th2-positive and Th2-negative bias in the upper airways of Caucasian and Chinese CRSwNP patients (10–12). We have previously demonstrated that while mucosal inflammation in Caucasian patients is mainly characterized by increased eosinophilic inflammation, in most Chinese patients, there is a lack of Th2 polarization characterized by a neutrophilic inflammation. Our findings of asthma comorbidity in the Chinese CRSwNP patient are also in accordance with recent findings (13) demonstrating that increased expression of IL-5 or total IgE and specific SE-IgE significantly increased the risk of asthma manifesting as a comorbid condition in patients with nasal polyposis, irrespective of ethnicity.

To our knowledge, this is the first study to demonstrate that CRSwNP patients with IL5-positive and IL-5-negative nasal polyps are more frequently colonized with bacteria than patients without nasal polyps and importantly that there is differential bacterial colonization, which appears to be associated with the absence or presence of IL-5 in the nasal polyps of these patients. The IL-5-positive group appears to be

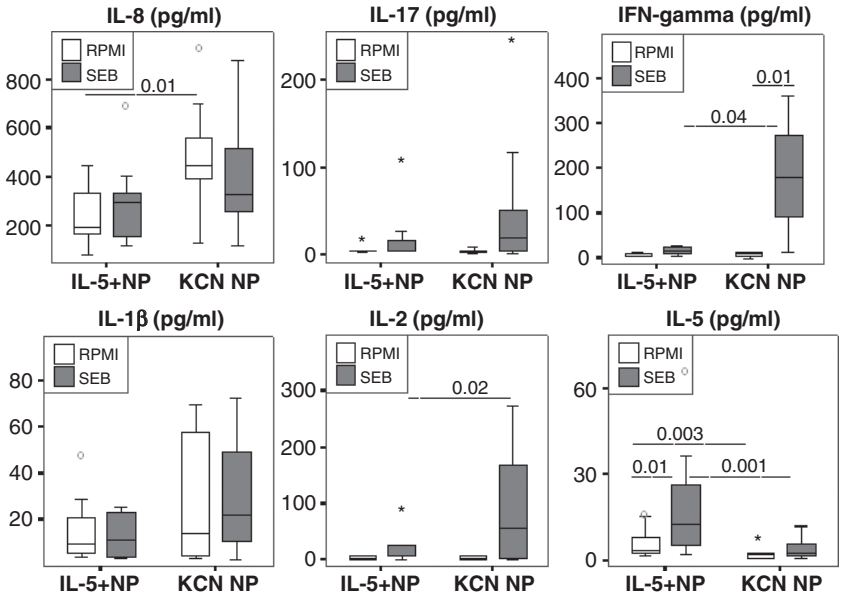

Figure 3 Effect of SEB stimulation on cytokine release from IL-5-positive and key cytokine-negative (KCN) nasal polyp tissue cultured for 24 h *in vitro* (the Kruskal–Wallis test for paired comparisons was used to assess intergroup variability. The Mann–Whitney *U* 2-tailed test was used for unpaired comparisons between groups. Significance level was set at ≤0.05). As expected, IL-5-positive polyps release IL-5, whereas KCN polyps release IFN and IL-2 upon stimulation and spontaneously release IL-8, confirming their neutrophilic pattern.

associated with a predominantly Gram-positive type bacterial colonization, whereas the IL-5 negative, particularly the KCN group, does not. Presently, the underlying mechanisms or the clinical implications of this association in CRSwNP patients are not clear; however, as *S. aureus* products have been demonstrated to activate both T and B lymphocytes, it is tempting to speculate that this may be involved in the modulation of immunoregulatory cell activity and/or innate immunity via toll-like receptors. Recent evidence indicates that SEs are able to induce the maturation and activation of dendritic cells via TLR2 signalling and that SE-pulsed dendritic cells drive the polarization of naive T cells into Th2 subsets (18–21). It has been suggested that activation of dendritic cells by SE leads to the synthesis of T-cell immunoglobulin mucin domain (TIM) 4, which subsequently interacts with TIM1 expressed on naive CD4 T cells and drives them to develop into Th2 cells (19).

In a third of the IL-5 + polyps, specific IgE antibodies to staphylococcal superantigens could be demonstrated, confirming previous data. In these patients, the presence of SE-IgE had the typical reflections on the expression of total IgE and markers of eosinophilic inflammation, which have been linked to increased asthma comorbidity (13). In contrast, the presence of SE-IgE in the neutrophilic nasal polyps was rare and did not lead to the typical eosinophilic bias.

In summary, our study has indicated that there are clear differences in the total bacterial load and type of bacterial species colonizing the nasal passages of Chinese patients with and without nasal polyps. Importantly, in CRSwNP patients, the absence of IL-5 expression in the nasal polyps appears to be associated with an increased colonization by Gram-negative bacteria *vs* controls, whereas the expression of IL-5 in the polyps is associated with predominantly Gram-positive bacterial colonization. The exact interrelation between these observations needs to be further evaluated; the bacterial type colonizing nasal polyps of CRSwNP patients may be determined by the inflammatory pattern in the polyps, as it was recently shown that alternative activation of macrophages in the Th2-biased milieu of nasal polyps may impact on the phagocytic and bacterial killing capacity of local macrophages (22). On the other hand, the predominant bacterial load may have an impact on the inflammatory pattern, as was observed in the gingival models of inflammation (23). We propose that the interaction of the microbiome with the mucosal immune response deserves our future scientific attention.

Acknowledgments

The authors thank Dr. Yi Xie and Prof Nannan Wang for providing the Vitek system and identification of the bacteria. The authors also thank Dr. Jagdish Devalia for his help in editing this manuscript.

Declaration of all sources of funding

Supported by the Program of Science and Technology Foundation of Sichuan Province (grant no. 2009HH0027) and the National Natural Science Foundation of China (grant no. 30973291) to Shixi Liu, by a collaboration between Ghent University and Sichuan University, by grants to Claus Bachert from the Flemish Scientific Research Board, FWO, Nr.

A12/5-HB-KH3, project G.0436.04, 3G.0489, G.0642.10N, by the Global Allergy and Asthma European Network (GA^{2}LEN), and by the Interuniversity Attraction Poles Program – Belgian State -29 Belgian Science Policy, Nr. IAP P6/35.

Conflict of interest

None.

References

1. Fokkens W, Lund V, Mullol J, European Position Paper on Rhinosinusitis and Nasal Polyps group. European position paper on rhinosinusitis and nasal polyps 2007. *Rhinol Suppl* 2007;**20**:1–136.
2. Platts-Mills TA, Rosenwasser LJ. Chronic sinusitis consensus and the way forward. *J Allergy Clin Immunol* 2004;**114**:1359–1361.
3. Bhattacharyya N, Lee LN. Evaluating the diagnosis of chronic rhinosinusitis based on clinical guidelines and endoscopy. *Otolaryngol Head Neck Surg* 2010;**143**:147–151.
4. Polzehl D, Moeller P, Riechelmann H, Perner S. Distinct features of chronic rhinosinusitis with and without nasal polyps. *Allergy* 2006;**61**:1275–1279.
5. Van Zele T, Claeys S, Gevaert P, Van Maele G, Holtappels G, Van Cauwenberge P et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. *Allergy* 2006;**61**:1280–1289.
6. Pérez-Novo CA, Claeys C, Van Cauwenberge P, Bachert C. Expression of eicosanoid receptors subtypes and eosinophilic inflammation: implication on chronic rhinosinusitis. *Respir Res* 2006;**7**:75.
7. Van Bruaene N, Pérez-Novo CA, Basinski TM, Van Zele T, Holtappels G, De Ruyck N et al. T-cell regulation in chronic paranasal sinus disease. *J Allergy Clin Immunol* 2008;**121**:1435–1441.
8. Van Bruaene N, Derycke L, Perez-Novo CA, Gevaert P, Holtappels G, De Ruyck N et al. TGF-beta protein and receptor expression, and intracellular signaling in chronic rhinosinusitis. *J Allergy Clin Immunol* 2009;**124**:253–259.
9. Pawankar R, Nonaka M. Inflammatory mechanisms and remodeling in chronic rhinosinusitis and nasal polyps. *Curr Allergy Asthma Rep* 2007;**7**:202–208.
10. Li X, Meng J, Qiao X, Liu Y, Liu F, Zhang N et al. Expression of TGF, matrix metalloproteinases, and tissue inhibitors in Chinese chronic rhinosinusitis. *J Allergy Clin Immunol* 2010;**125**:1061–1068.
11. Zhang N, Van Zele T, Perez-Novo C, Van Bruaene N, Holtappels G, DeRuyck N et al. Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease. *J Allergy Clin Immunol* 2008;**122**:961–968.
12. Zhang N, Holtappels G, Claeys C, Huang G, van Cauwenberge P, Bachert C. Pattern of inflammation and impact of *Staphylococcus aureus* enterotoxins in nasal polyps from southern China. *Am J Rhinol* 2006;**20**:445–450.
13. Bachert C, Zhang N, Holtappels G, De Lobel L, van Cauwenberge P, Liu S et al. Presence of IL-5 protein and IgE antibodies to staphylococcal enterotoxins in nasal polyps is associated with comorbid asthma. *J Allergy Clin Immunol* 2010;**126**:962–968.
14. Fokkens W, Lund V, Mullol J. EP3OS 2007: European position paper on rhinosinusitis and nasal polyps 2007. A summary for otorhinolaryngologists. *Rhinology* 2007; **45**:97–101.
15. Patou J, Gevaert P, Van Zele T, Holtappels G, van Cauwenberge P, Bachert C. *Staphylococcus aureus* enterotoxin B, protein A, and lipoteichoic acid stimulations in nasal polyps. *J Allergy Clin Immunol* 2008;**121**: 110–115.
16. Niederfuhr A, Kirsche H, Riechelmann H, Wellinghausen N. The bacteriology of chronic rhinosinusitis with and without nasal polyps. *Arch Otolaryngol Head Neck Surg* 2009;**135**:131–136.
17. Qiao F, Xie Y, Yin WJ, Kang M, Guo XJ, Chen HJ et al. Nasal colonization by opportunistic pathogens among health care workers: a survey. *Chin J Nosocomiol* 2008;**18**:1371–1373.
18. Mandron M, Ariès MF, Boralevi F, Martin H, Charveron M, Taieb A et al. Age-related differences in sensitivity of peripheral blood monocytes to lipopolysaccharide and *Staphylococcus aureus* toxin B in atopic dermatitis. *J Invest Dermatol* 2008;**128**:882–889.
19. Liu T, He SH, Zheng PY, Zhang TY, Wang BQ, Yang PC. Staphylococcal enterotoxin B increases TIM4 expression in human dendritic cells that drives naive CD4 T cells to differentiate into Th2 cells. *Mol Immunol* 2007;**44**:3580–3587.
20. Mandron M, Ariès MF, Brehm RD, Tranter HS, Acharya KR, Charveron M et al. Human dendritic cells conditioned with *Staphylococcus aureus* enterotoxin B promote TH2 cell polarization. *J Allergy Clin Immunol* 2006;**117**:1141–1147.
21. Chau TA, McCully ML, Brintnell W, An G, Kasper KJ, Vinés ED et al. Toll-like receptor 2 ligands on the staphylococcal cell wall downregulate superantigen-induced T-cell activation and prevent toxic shock syndrome. *Nat Med* 2009;**15**:641–648.
22. Krysko O, Holtappels G, Zhang N, Kubica M, Deswarte K, Derycke L et al. Alternatively activated macrophages and impaired phagocytosis of *S. aureus* in chronic rhinosinusitis. *Allergy* 2010;**66**:396–403.
23. Ohlrich EJ, Cullinan MP, Seymour GJ. The immunopathogenesis of periodontal disease. *Aust Dent J* 2009;**54**(Suppl 1):S2–S10.