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Vγ1+ γδT Cells Are Correlated With Increasing Expression of Eosinophil Cationic Protein and Metalloproteinase-7 in Chronic Rhinosinusitis With Nasal Polyps Inducing the Formation of Edema

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Purpose: We have found that expression of γδT cells is increased in pathological mucosa of chronic rhinosinusitis with nasal polyps (CRSwNP) compared with normal nasal mucosa. This increase is correlated with the infiltration of eosinophils in CRSwNP. Here, we investigated the expression of γδT cells, inflammation and tissue remodeling factors as well as their probable relationships in different types of chronic rhinosinusitis (CRS) in China. **Methods:** A total of 76 surgical tissue samples that included 43 CRSwNP samples (15 eosinophilic and 28 non-eosinophilic), 17 CRS samples without nasal polyps (CRSsNP), and 16 controls were obtained. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) was used to measure the mRNA expression levels of Vγ1+ γδT cells, Vγ4+ γδT cells, eosinophil cationic protein (ECP), interleukin (IL)-8, transforming growth factor (TGF)-β2, metalloproteinase (MMP)-7, tissue inhibitor of metalloproteinase (TIMP)-4 and hypoxia-inducible factor (HIF)-1α. Enzyme linked immunosorbent assay (ELISA) was used to measure the protein level of ECP and MMP-7 in CRSwNP. The eosinophils were counted and the level of edema was analyzed with HE staining. **Results:** The mRNA expression levels of the Vγ1 subset, ECP and MMP-7 were significantly increased in CRSwNP with histological characteristics of eosinophilic infiltration and edema. The expression of the Vγ1 gene in CRSwNP correlated positively with the expression of both ECP and MMP-7. No significant decreases in the mRNA expression levels of TGF-β2, TIMP-4 or HIF-1α were observed in the CRSwNP samples. The expression levels of Vγ1 gene, ECP and MMP-7 were significantly increased in eosinophilic CRSwNP compared to non-eosinophilic CRSwNP. **Conclusions:** Our results suggest the associations between Vγ1+ γδT cells, ECP and MMP-7 in CRSwNP, indicating that Vγ1+ γδT cells can induce the eosinophilic inflammation, which has a further effect on the formation of edema.

Key Words: Chronic rhinosinusitis; nasal polyps; γδT cells; inflammation; tissue remodeling

INTRODUCTION

Chronic rhinosinusitis (CRS) is one of the most common chronic diseases, with an estimated prevalence of 12% in the United States.[1] CRS has a substantial influence on the quality of life and is a burden to the social economy.[2] Clinically, there are 2 types of CRS: chronic rhinosinusitis without nasal polyps (CRSsNP) and chronic rhinosinusitis with nasal polyps (CRSwNP). These different types of CRS have distinct inflammatory features and tissue remodeling patterns. CRSsNP is characterized predominantly by a T-helper type 1 (Th1)-induced neutrophilic inflammation with elevated expressions of transforming growth factor (TGF)-β1 and tissue inhibitors of metalloproteinase (TIMPs), which result in remodeling that includes excessive extracellular matrix (ECM) deposition, interstitial fibrosis, basement membrane thickening and goblet cell hyperplasia without obvious pseudocyst formation.[3] CRSwNP is featured by a T-helper type 2 (Th2)-skewed eosinophilic inflammation that involves prominent edema formation and a decreased level of TGF-β1, which lead to fibrosis and increased levels of metalloproteinases (MMPs), which in turn lead to pseudocyst formation.[3] According to the inflammatory pattern, CRSwNP is further divided into eosinophilic CRSwNP (Eos CRSwNP) and non-eosinophilic CRSwNP (non-Eos CRSwNP). There are differences between the two subtypes of CRSwNP.

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Non-Eos CRSwNP is more likely to involve neutrophilic inflammation and fibrosis and shares some of the characteristics of both CRSsNP and Eos CRSwNP.[3] The exact mechanisms responsible for the differences remain unclear.

γδT cells are a small but distinctive subset of T cells that were first recognized by Brenner in 1986.[4] These cells primarily colonize in the mucosa and skin where they play an important role in immune regulation.[4] γδT cells have been suggested to mediate inflammatory cell infiltration and remodeling in a murine model via interleukin (IL)-17A, which has been proven to be a crucial regulator of cardiac fibrosis.[5-8] However, the links between γδT cells, inflammation and tissue remodeling in CRS appear to be unknown.

In our previous study, we found that the expression of γδT cells is elevated in the pathological mucosa of CRSwNP compared with normal nasal mucosa and that γδT cell expression is positively correlated with the infiltration of eosinophils in Eos CRSwNP.[9]

Previous studies have shown that different subsets of γδT cells play distinct roles. The Vγ1 subset has been demonstrated to promote inflammation particularly due to the enhancement of eosinophilic infiltration via Th2 cytokines and airway hyperresponsiveness (AHR).[10] The Vγ4 subset has been demonstrated to suppress inflammation.[10] Our studies have indicated that Vγ1$^+$ γδT cells might be a dominant subset in the nasal mucosa of CRSwNP.[9] However, whether similar mechanisms are responsible for the pathogenesis of CRS or not remains unclear. Thus, the present work aimed to investigate the relationships between γδT cells, particularly the Vγ1 subset, inflammation and tissue remodeling in different types of CRS.

MATERIALS AND METHODS

Subjects and study design

A total of 76 patients were selected from the inpatients (between 2011 and 2014) of the Department of Otolaryngology-Head and Neck Surgery, the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. The subjects included 17 with CRSsNP, 43 with CRSwNP (15 with Eos CRSwNP and 28 with non-Eos CRSwNP), and 16 control subjects. Detailed characteristics of these subjects are provided in Table 1. The diagnoses and classification of CRS were based on the European Academy of Allergy and Clinical Immunology (EAACI) guidelines (EPOS2012).[11] Patients with allergic diseases, fungal rhinosinusitis, or severe pulmonary and cardiac diseases were excluded from this study. None of the enrolled patients had histories of surgery or the use of corticosteroids or antibiotics within the month prior to surgery. The nasal polyps (NPs), diseased ethmoid sinus mucosa or uncinate processes of the patients with CRS were collected during functional endonasal sinus surgery as the case group. The normal nasal mucosa of the inferior turbinate, ethmoid sinus or uncinate process were taken from the patients with deviations of the nasal septum, cerebrospinal fluid rhinorrhea or traumatic optic neuropathy who presented with no sinus inflammation as the control group. All tissue samples were stored as duplicates at -80°C after rapid freezing in liquid nitrogen; one sample was used for quantitative reverse transcription-polymerase chain reaction (qRT-PCR), and the other was used for histological analysis. CRSwNP was regarded as eosinophilic when the percentage of eosinophils in the tissue exceeded 10% of total infiltrating cells as mentioned elsewhere.[12]

Table 1. Clinical data of the subjects

Characteristics	Control	CRSsNP	Non-Eos CRSwNP	Eos CRSwNP
Subjects, n	16	17	28	15
Age (yr), mean±SD	34.2±16.0	40.9±15.9	33.1±16.8	42.7±13.8
Sex: female/male, n	8/8	5/12	6/22	7/8
Smoking, n (%)	2 (12.5)	5 (29.4)	7 (25.0)	3 (20.0)
CT score (Lund-Mackay), mean±SD	0.8±1.7	8.1±3.1	11.9±7.0	12.3±6.4

CRS, chronic rhinosinusitis; CRSsNP, CRS without nasal polyps; non-Eos CRSwNP, non-eosinophilic CRS with nasal polyps; Eos CRSwNP, eosinophilic CRSwNP; n, number; SD, standard deviation; CT, computed tomography.

This study was approved by the Ethical Committee of the Third Affiliated Hospital of Sun Yat-sen University (File No. [2013] 2-9). All patients were informed of the purposes and procedures of the study and provided written informed consent.

Quantitative RT-PCR

The total RNA of each tissue sample was extracted using RNAiso Plus (TaKaRa, Dalian, Japan) and reverse-transcribed to cDNA with random hexamer primers and RT-PCR kits (TaKaRa DDR036S, Dalian, Japan). The Vγ1 and Vγ4 subsets of γδT cells and the levels of eosinophil cationic protein (ECP), IL-8, TGF-β2, MMP-7, TIMP-4, hypoxia-inducible factor (HIF)-1α cytokines were detected. PCR was performed with an ABI 7500 FAST instrument (Foster City, CA, USA) using a SYBR Premix Ex Taq kit (TaKaRa DDR420A, Dalian, Japan) with the appropriate primers (Table 2) as reported elsewhere.[3,13,14] The relative gene expressions were calculated with the comparative CT method. β2 microglobulin (β2M) was used as the housekeeping gene for normalization, and a no-template sample was used as the negative control.

Elisa for ECP and MMP-7 in CRSwNP

Tissue samples were grinded in mortar with liquid nitrogen, then each tissue dry powder was weighed, and 100 μL PBS supplemented with 10% protease inhibitor cocktail (MedChem Express, Shanghai, China) was added per 10 mg tissue. After homogenization, the suspensions were centrifuged at 3,000 rpm for 15 minutes at 4°C. Supernatants were separated and stored

Table 2. Primers for quantitative RT-PCR analysis

Gene	Sequence
Vγ1	(F)TACCTACACCAGGAGGGGAAG
Vγ4	(F)GATTGCTCAGGTGGGAAGACT
Cγ	(R)GTTGCTCTTCTTTTCTTGCC
ECP	(F)5′-TCGGAGTAGATTCCGGGTG-3′
	(R)5′-GAACCACAGGATACCGTGGAG-3′
IL-8	(F)5′-GAATGGGTTTGCTAGAATGTGATA-3′
	(R)5′-CAGACTAGGGTTGCCAGATTTAAC-3′
TGF-β2	(F)5′-CTCCTACAGACTTGAGTCACAAC-3′
	(R)5′-CTCCATAAATACGGGCATGCTCC-3′
MMP-7	(F)5′-GAGGCATGAGTGAGCTACAGTG-3′
	(R)5′-CATCTCCTTGAGTTTGGCTTCT-3′
TIMP-4	(F)5′-TTTTGACTCTTCCCTCTGTGGT-3′
	(R)5′-CTGTGTAGCAGGTGGTGATTTG-3′
HIF-1α	(F)5′-CAGCCGCTGGAGACACAATC-3′
	(R)5′-TTTCAGCGGTGGGTAATGGA-3′
β2M	(F)5′-TACACTGAATTCACCCCCAC-3′
	(R)5′-CATCCAATCCAAATGCGGCA-3′

RT-PCR, reverse transcription-polymerase chain reaction; Vγ1, Vγ4, subsets of γδT cells; Cγ, reverse sequence of both Vγ1 and Vγ4; ECP, eosinophil cationic protein; IL-8, interleukin-8; TGF-β2, transforming growth factor-β2; MMP-7, metalloproteinase-7; TIMP-4, tissue inhibitor of metalloproteinase-4; HIF-1α, hypoxia-inducible factor-1α; β2M, β2 microglobulin.[3,13,14]

at -80°C until analyzed.

ECP (Cusabio Biotech, CSB-E11729h, Wuhan, China) and MMP-7 (OriGene Technologies, EA100322, Beijing, China) levels were measured with commercially available ELISA kits. The minimal detection limits for these kits are 1.560 and 0.156 ng/mL, respectively. All procedures followed the manufacturer's recommendations. Concentrations of ECP and MMP-7 in the tissue homogenate were normalized to the concentration of total protein.

Histological analysis

For histological analysis, each sample was fixed in 10% neutral buffered formalin and subsequently embedded in paraffin. Paraffin sections (5 mm) were stained with hematoxylin and eosin (HE) dyes. All of the stained sections were examined by 2 independent physicians who were blind to the clinical data. Eosinophils were counted under high-power (HP) magnification (400-fold). Five fields per sample were randomly selected for analysis. The sample was regarded as Eos CRSwNP when the percentage of eosinophils exceeded 10% of the total infiltrating cells.[12] The HE-stained sections were used to determine the general pathologic features at 200-fold magnification. According to Hellquist's classification, each tissue sample was classified as edematous, glandular hyperplastic, fibrotic, or atypical based on the remodeling features.[15] A 3-point scale was used to semiquantitatively score the level of edema; 0 represented the least amount of edema, and 2 indicated the most extensive edema.[16]

Statistical analysis

The statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA). For the continuous variables, the results are expressed as the medians and interquartile ranges or with Tukey box-and-whisker plots. The Kruskal-Wallis H-test was used to assess the significance of intergroup variability. Two-tailed Mann-Whitney *U* tests were used for between group comparisons. The differences in proportions between groups were evaluated with χ^2 tests. Bonferroni corrections were applied to adjust for multiple comparisons (*i.e.*, $\alpha=0.05/3=0.016$). Spearman tests were used to examine the correlations. The level of significance was set at $P<0.05$.

RESULTS

The expression of Vγ1 gene was elevated in the CRSwNP samples

Vγ1 and Vγ4 genes were detected in this study, and the differences between the groups were significant ($P=0.005$). The expression of Vγ1 gene in the CRSwNP samples was significantly greater than those in the CRSsNP and control samples, and no significant difference between the CRSsNP and control samples was observed (Fig. 1A). Furthermore, as illustrated in Fig. 1B, no significant differences of Vγ4 gene were observed between the groups.

The expression of ECP was elevated in CRSwNP

Fig. 1C illustrates the relative expression levels of ECP in the different groups. This level was significantly increased in the CRSwNP samples, and a significant difference between groups was detected ($P=0.002$). Moreover, the expression of IL-8 varied significantly between groups ($P=0.008$), and the difference between the CRSsNP and control groups was significant (Fig. 1D). No significant differences between the other groups were observed.

The expression of MMP-7 was increased in the CRSwNP samples

Similarly, significant differences in MMP-7 expression between groups were detected ($P=0.003$). The relative expression level of MMP-7 in the CRSwNP samples was significantly greater than those of the other two groups (Fig. 1E). Nevertheless, no significant differences were observed when we compared the relative mRNA expression levels of TGF-β2, TIMP-4, and HIF-1α across the groups (Fig. 1F-H).

CRSwNP was primarily characterized by correlated eosinophils infiltration and edema

The inflammatory type of CRSwNP was proven to be associated with eosinophilic infiltration compared to CRSsNP (Fig. 2).

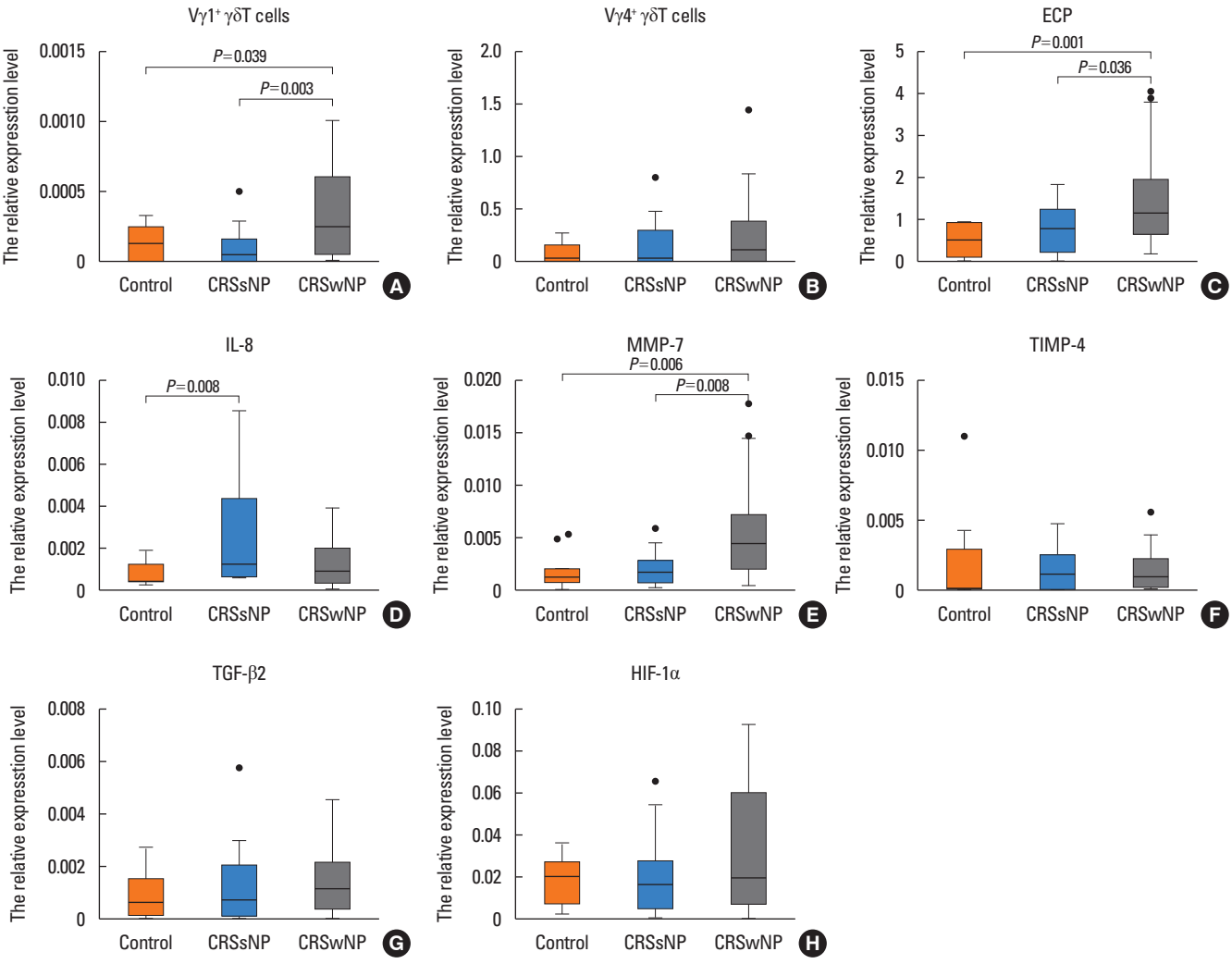

Fig. 1. The relative mRNA expression levels for Vγ1+ γδT cells, Vγ4+ γδT cells, ECP, IL-8, TGF-β2, MMP-7, TIMP-4, and HIF-1α. Vγ1, Vγ4, subsets of γδT cells; ECP, eosinophil cationic protein; IL-8, interleukin-8; TGF-β2, transforming growth factor-β2; MMP-7, metalloproteinase-7; TIMP-4, tissue inhibitor of metalloproteinase-4; HIF-1α, hypoxia-inducible factor-1α.

First, a significant between-group difference was detected ($P<0.001$). The eosinophils accounted for a greater percentage of the total inflammatory cells in the CRSwNP samples than in the CRSsNP samples ($P=0.038$) and the control samples ($P<0.001$), and the percentage of eosinophils in the CRSsNP samples was also significantly greater than in the control samples ($P<0.001$; Fig. 3B). Additionally, the proportion of Eos CRSwNP in the CRSwNP samples was 34.9% (15/43), and the proportion of non-Eos CRSwNP was 65.1% (28/43).

The semiquantitative edema scores were also assessed. The edema scores of the CRSwNP and CRSsNP samples were significantly greater than those of the control samples ($P<0.001$ and $P=0.001$, respectively), and the scores for the CRSwNP samples were greater than those for the CRSsNP samples, although this difference was not statistically significant (Fig. 3A).

Positive correlations were observed between the percentages of eosinophils and edema scores ($r_s=0.482$, $P<0.001$).

According to the abovementioned Hellquist's classification, the majority of the histological patterns observed in the CRS tissue samples were fibrotic and edematous types, a small number of samples showed the glandular hyperplastic type, and none of the samples showed the atypical type (Fig. 4). The edematous type was more frequently observed in the CRSwNP samples (46.5%) than the CRSsNP samples (29.4%). The proportion of edematous-type CRSwNP was similar to that of fibrotic-type samples (51.2%). Moreover, the primary type of CRSsNP was the fibrotic one (58.8%). When the CRSwNP samples were further classified as non-Eos CRSwNP and Eos CRSwNP according the number of eosinophils as previously mentioned, the edematous type was found to be more frequent among the Eos CRSwNP samples (66.7%) than the non-Eos CRSwNP (35.7%) and CRSsNP samples. The percentage of fi-

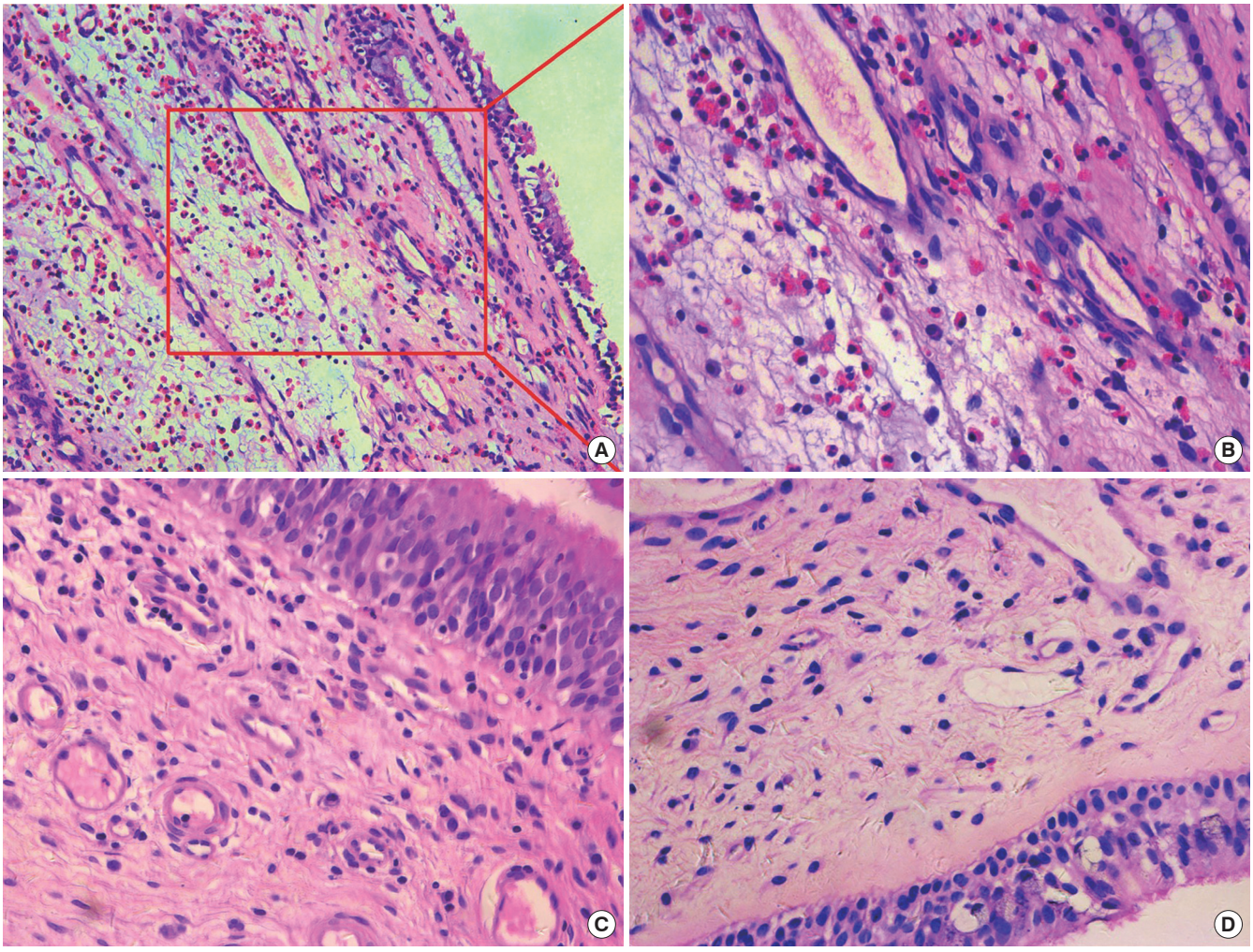

Fig. 2. A large amount of eosinophil infiltration in the subepithelial tissue of a CRSwNP sample compared to CRSsNP and control (HE-stained section). (A) CRSwNP: epithelial cell damage and basement membrane thickness (200× magnification). (B) CRSwNP: eosinophils scattered in the subepithelial tissue with pseudocyst formation and edema, a lack of collagen and the disappearance of blood vessels and glands (400× magnification). (C) CRSsNP: lymphocytes and neutrophils scattered with increased collagen and fibrosis (400× magnification). (D) Control: normal nasal mucosa (400× magnification of a control sample). CRSwNP, chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis samples without nasal polyps; HE, hematoxylin and eosin.

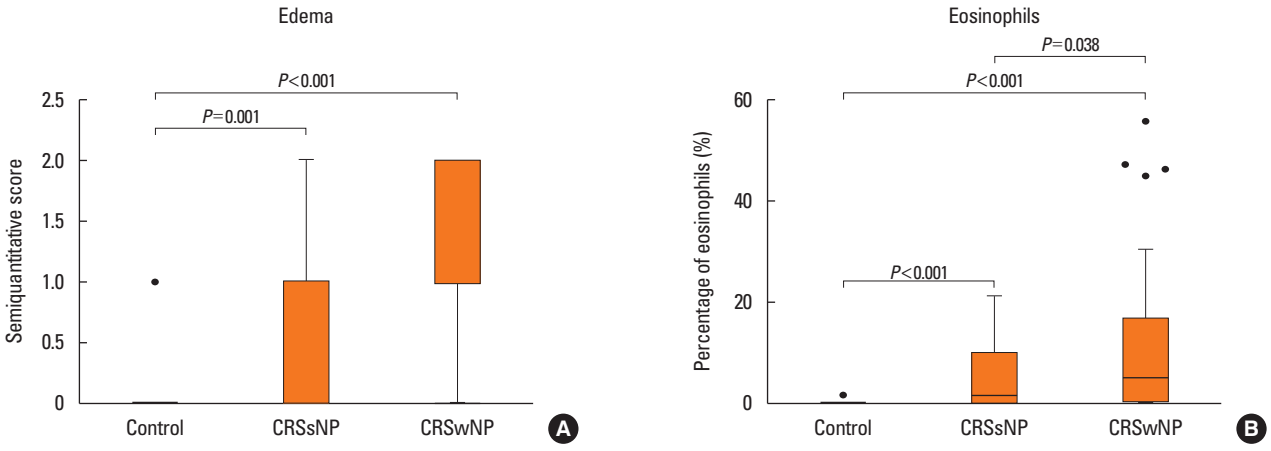

Fig. 3. Semiquantitative edema scores and eosinophil infiltrations in the different CRS types and controls. (A) Edema: the semiquantitative score of the severity of edema. (B) Eosinophil: the percentage of eosinophils relative to the total inflammatory cells. CRS, chronic rhinosinusitis.

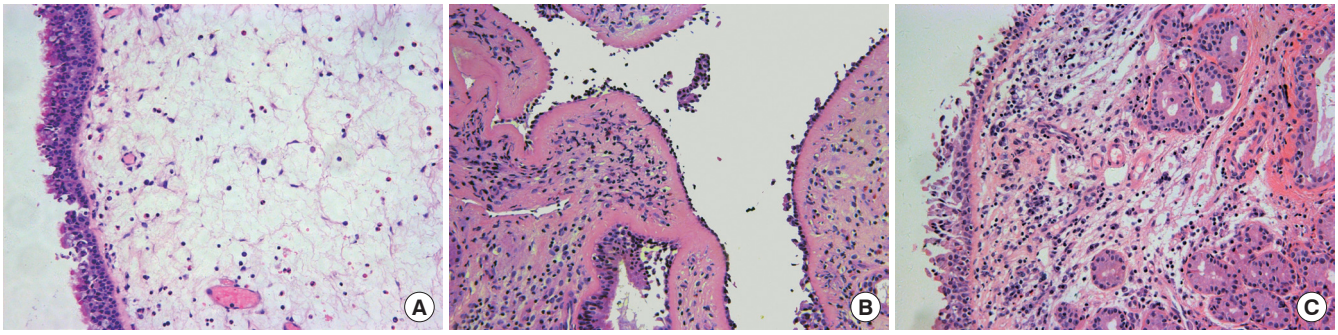

Fig. 4. Hellquist's classification of CRS. HE-stained tissue sections at 200× magnification. (A) Edematous type: subepithelial edema, a lack of collagen, and the disappearance of glands and blood vessels with inflammatory cell infiltration. (B) Fibrotic type: thickened basement membrane, subepithelial collagen deposition, and inflammatory cell infiltration. (C) Glandular hyperplastic type: increased amounts of glands and blood vessels. CRS, chronic rhinosinusitis; HE, hematoxylin and eosin.

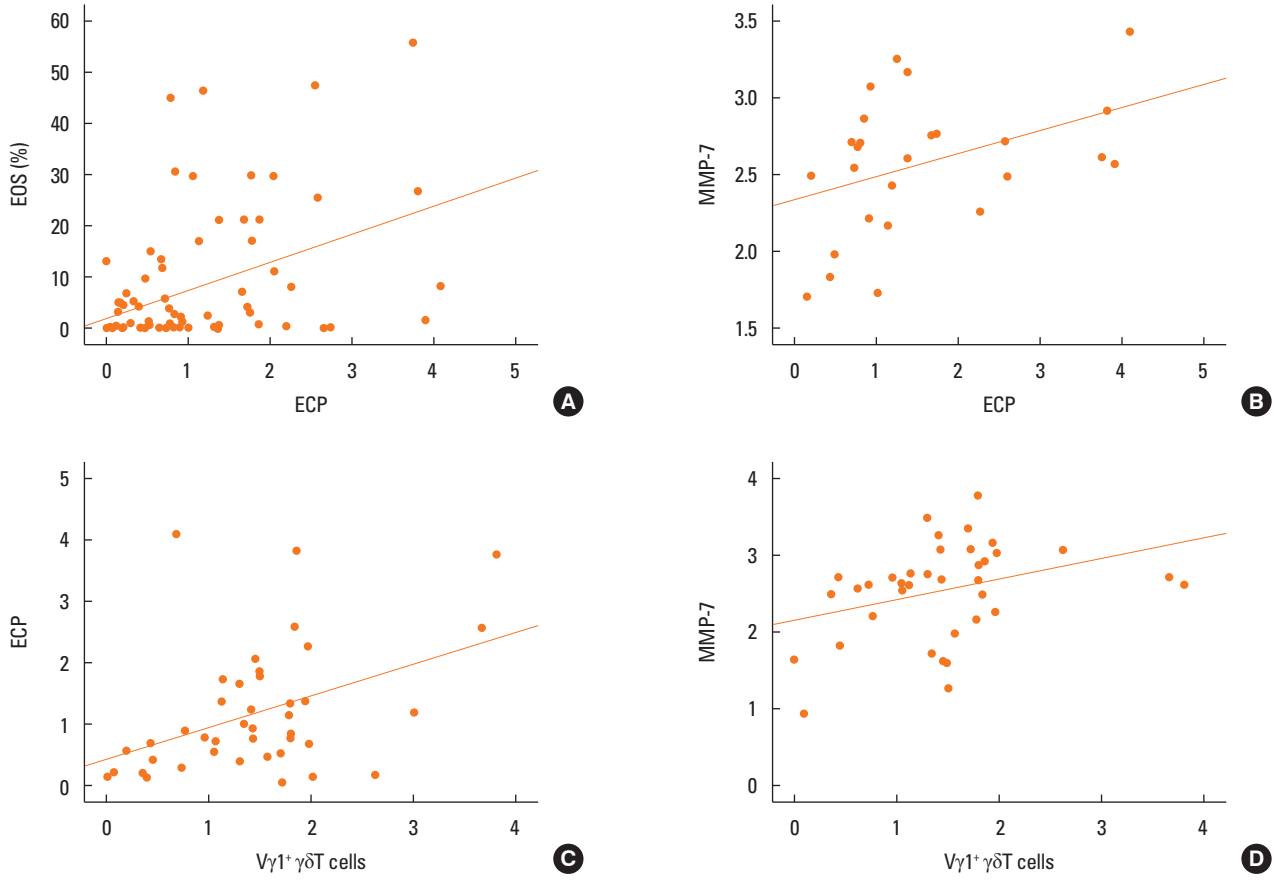

Fig. 5. Correlations between γδT cells, inflammation and remodeling. (A) The correlation coefficient for the relationship of Eos and ECP: $r_s=0.386$, $0.011 \leq P<0.05$. (B) The correlation coefficient for the association between MMP-7 and ECP: $r_s=0.418$, $0.030 \leq P<0.05$. (C) The correlation coefficient for the association between ECP and Vγ1⁺ γδT cells: $r_s=0.368$, $0.019 \leq P<0.05$. (D) The correlation coefficient for the association between MMP-7 and Vγ1⁺ γδT cells: $r_s=0.353$, $0.032 \leq P<0.05$. The data (x) regarding the relative mRNA expression levels of ECP, MMP-7 and Vγ1⁺ γδT cells were transformed with same equation $\{x'=\log_{10}(10^5x+1)\}$. Eos, eosinophils; ECP, eosinophil cationic protein; MMP-7, metalloproteinase-7.

brotic-type Eos CRSwNP (33.3%) was much lower than that of the other 2 groups; *i.e.*, the non-Eos CRSwNP (60.7%) and CRSsNP more frequently presented as the fibrotic type. There were no significant differences between the groups.

The relationships of γδT cells, inflammation and remodeling

The correlations between the percentage of eosinophils and the mRNA expression level of ECP, as well as the mRNA expression levels of ECP and MMP-7 were examined to identify positive correlations, and the results are illustrated in Fig. 5. Similar

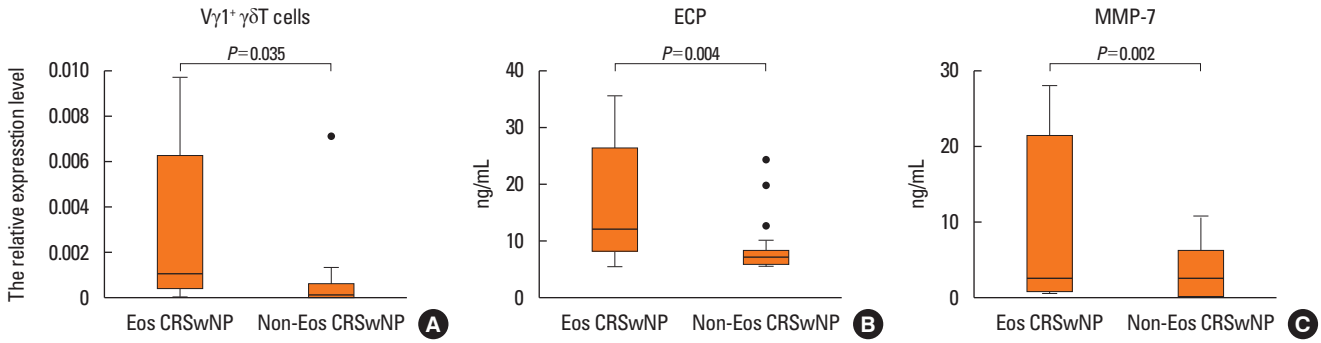

Fig. 6. The relative mRNA expression levels for (A) Vγ1+ γδT cells, (B) the protein level of ECP, and (C) MMP-7 in Eos CRSwNP compared to non-Eos CRSwNP. ECP, eosinophil cationic protein; MMP-7, metalloproteinase-7; CRSwNP, chronic rhinosinusitis with nasal polyps; non-Eos CRSwNP, non-eosinophilic CRSwNP.

outcomes were obtained when the same analysis was applied to Vγ1+ γδT cells, ECP and MMP-7. The relative expression level of Vγ1 gene was positively correlated with those of ECP and MMP-7.

The expression level of Vγ1 gene, ECP and MMP-7 in Eos CRSwNP and non-Eos CRSwNP

The relative mRNA expression level for Vγ1+ γδT cells was significantly increased in Eos CRSwNP compared to non-Eos CRSwNP. Moreover, the protein levels of both ECP and MMP-7 in Eos CRSwNP were significantly increased compared to non-Eos CRSwNP (Fig. 6).

DISCUSSION

Based on the differential expression of the T cell receptor (TCR), T cells are divided into αβT and γδT cell subsets. αβT cells are primarily peripheral blood mononuclear cells, and γδT cells account for less than 10% (approximately 3%-5%) of all lymphoid cells in the secondary lymphoid tissues and blood.[17] γδT cells are a distinctive subset of T cells. Nevertheless, γδT cells are much more prevalent in mucosal and epithelial sites. In these sites, γδT cells comprise as much as 50% of the total intraepithelial lymphocyte populations.[18] These cells play an important role in innate immune responses, which ultimately lead to adaptive immune responses. These cells are the first immune cells that are found in the fetus and provide immunity to newborns prior to the activation of the adaptive immune system.[19] Subsets of γδT cells have been found in both mice and humans. The distinct subsets of γδT cells with different γ and δ TCR chains that colonize specific epithelial locations in diseases of the lungs, intestines, skin, uterus, vagina and tongue need to be identified.[17,20-24] As the epithelial cells of nasal mucosa is the first mechanical barrier of the airway, it has a great effect on the mucosal immune system. Therefore, γδT cells are likely to act on the pathological process of CRS.

Distinct histological features were present in different types of CRS. Previous studies based on Caucasian patients have suggested that CRSwNP is primarily characterized by eosinophilic inflammation and edematous remodeling patterns.[2,25] However, this situation is different from that of Asian patients. Recently, a few studies based on Asians have shown that minor eosinophilic inflammation accounts for the distinctive features of CRSwNP.[16,26,27] Moreover, the expanding racial differences that have been reported indicate that Caucasian patients with CRSwNP and comorbid asthma are more common than Chinese patients with both conditions.[28] Studies have shown that the NP samples from Caucasians exhibit Th2-skewed and eosinophilic inflammation patterns, and NP samples from Chinese people exhibit both Th1/Th17 and Th2 cell patterns with neutrophilic and eosinophilic inflammation.[12,16,27] In the present study, the CRSwNP samples from Chinese patients also exhibited similar characteristics and a greater proportion of non-eosinophilic inflammatory patterns. Moreover, it is noteworthy that evidence indicates that γδT cells participate in Th2 immune responses. In contrast, the available data suggest that a small fraction of γδT cells act as effective suppressors.[10,29,30] Taken together, distinct subsets of γδT cells likely produce certain cytokines that induce the infiltration of different inflammatory cells, such as eosinophils and neutrophils. However, the functions and capacities are not yet clear.

Correspondingly, our previous reports proved that γδT cell numbers are increased in CRSwNP pathological mucosa and positively correlated with the infiltration of eosinophils. The Vγ1 gene expression is also increased in CRSwNP pathological mucosa.[9] A study conducted by Hahn *et al.*[10] demonstrated similar results; these authors found that Vγ1+ γδT cells appeared to strongly enhance AHR and airway inflammation, whereas Vγ4+ γδT cells unambiguously suppressed AHR. The mRNA expression of Vγ1+ γδT cells was proven to positively correlate with that of ECP in CRSwNP in the present study, but the expression of Vγ4+ γδT cells was not found to be negatively correlated with either ECP or eosinophils. Again, these findings support the hypothesis that distinct subsets of γδT cells can be inhibitors or enhancers depending on the specific γ and δ TCR chains. Additional support for this notion is provided by the increasing

number of studies based on murine models or humans that suggest that γδT cells mediate inflammatory cell infiltration and remodeling in many diseases, such as liver inflammation and fibrosis, cardiac fibrosis, lung inflammation, asthma, allergic rhinitis, psoriasis and atopic dermatitis.[5,6,24,31,32] However, studies of the relationships of γδT cells, inflammation and remodeling in CRS are scarce. Hence, additional observations and data are needed to address this question.

Tissue remodeling is a dynamic process that is closely related to the extracellular matrix (ECM). If the balance between the formation and degradation of the ECM is disrupted, the rebuilding process occurs. When the body is attacked by exogenous or endogenous stimulation, the immune system promotes the accumulation and production of inflammatory cells including eosinophils, which are activated to release a variety of proteins and cytokines, including ECP, TGF-β, MMPs, TIMPs and ILs. Eosinophils and ECP have been clearly suggested to initiate the formation of edema. Flood-Page *et al.*[33] showed that anti-IL-5 treatment reduces eosinophils and fibrosis in the airways of asthmatics. Moreover, TGF-β has been indicated to be a key regulator of ECM production and tissue remodeling, whereas MMPs are known to be important regulators of the degradation of the ECM. Matrilysin MMP-7 is upregulated, which disturbs the tissue homeostasis and progresses to the formation of edema. TIMPs act as inhibitors of MMPs. Finally, all of these confounding and multiple regulators may ultimately contribute to tissue remodeling.[3,34] Eosinophilic inflammation with the index by ECP can lead to the downregulation of TGF-β1 and TGF-β2, and the decreased expression of TIMP-4. Without the inhibitor of MMPs, the activity of MMP-7 and MMP-9 increased, which promoting degradation of ECM and formation of edema.[3,12,35] And vice versa, neutrophilic inflammatory may release IL-8 and lead to the increased expression of TGF-β1 and TGF-β2, as well as TIMP-4, which finally result in the deposition of collagen and fibrosis.[3,35] Moreover, studies have shown that under anoxic conditions, HIF-1α distributed in the airway of patients can be activated and lead to epithelial-to-mesenchymal transition.[36]

The current study revealed that the general histological features of CRSwNP are characterized by correlated edema and eosinophil infiltration, with the increased expressions of $V\gamma1^+$ γδT cells, ECP, and MMP-7, and a positive correlation between the two. No significant difference in the expressions of $V\gamma4^+$ γδT cells was observed in the different groups. As previously mentioned, Vγ4 mRNA expression should have decreased. The failure to observe this phenomenon might have been due to the patients themselves or the progression of chronic disease. In long-term CRS, the body has defended against the course of disease and has received some treatment. Furthermore, no significant increases in the mRNA expression levels of TGF-β2, TIMP-4 or HIF-1α were found in the CRSsNP samples in this study, and these results are inconsistent with the results of other studies.[3,35] Because the mRNA expression levels of these regulators in mucosa are minimal, and the majority of the CRSwNP samples belonged in the non-eosinophilic subset, there were no substantial differences between the CRSwNP and CRSsNP groups.

Thus, these results indicate that γδT cells may play a role in the mechanism of CRS pathogenesis which should not to be ignored. Moreover, these distinct cells appear to mediate eosinophil infiltration and eventually lead to the formation of edema in CRS. However, the detailed mechanisms remain unclear.

It has been suggested that γδT cells are activated in the presence of pathogens, antigens and stimulation by damage-associated molecular patterns (DAMPs) because macrophages promote NF-κB signaling that results in the release of IL-23, which is recognized by the corresponding receptor IL-23R of the γδT cells and activates nuclear translocator RORγt to produce IL-17A. This process leads to inflammatory cell infiltration or recruitment, which eventually causes edema or fibrosis.[5,6,37] IL-17A has been suggested to induce MMPs secretion.[36] Several studies have demonstrated that IL-17A upregulates Th2 cell-mediated eosinophilic airway inflammation and plays an important role in the remodeling of nasal polyps.[37,38] Additional evidence found in our previous study indicated that the expression of IL-17A is upregulated in CRSwNP. However, the role and the related mechanisms of IL-17A in remodeling have not been fully elucidated. Therefore, all of the current studies have demonstrated the potential significant mechanism by which γδT cells are related to inflammation and remodeling in CRS. In addition to benefiting scientific research, these findings provide another pathway for the management and intervention for CRS patients. However, the mechanisms underlying IL-17A production and the roles of IL-17A in inflammation and the early damage and remodeling of the mucosa in CRS remain unclear.

The significance of γδT cells in the pathogenesis of CRS and their effects on inflammation and remodeling require further investigations and evidences in murine models and humans.

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