

Responses of Nasal Polyp-Derived Macrophages to Inflammatory Cues and Potential Pathological Roles in Type 2 Chronic Rhinosinusitis With Nasal Polyps

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Objectives. The role of highly plastic and heterogeneous macrophages in the pathogenesis of nasal polyps (NP) is not well understood. We aimed to investigate the distribution and responses of NP-derived macrophages to inflammatory cues according to endotypes of chronic rhinosinusitis with nasal polyps (CRSwNP).

Methods. The distribution of macrophage subsets was analyzed using NP bulk RNA sequencing data with CIBERSORTx program. The cytokines and chemokines produced by NP-derived macrophages following stimulation with interleukin (IL)-4, staphylococcal enterotoxin B (SEB), and lipopolysaccharide were evaluated using a multiplex cytokine assay. Immunofluorescence staining in NP tissues was conducted to validate the findings.

Results. Type 2 CRSwNP exhibited a significantly higher proportion of M2 macrophages, whereas both M1 and M2 macrophage populations were elevated in non-type 2 CRSwNP. Baseline cytokine analysis of NP-derived macrophages revealed an increase in thymic stromal lymphopoietin (TSLP) levels in CRSwNP compared to controls. Furthermore, TSLP expression was significantly upregulated from baseline levels following IL-4 or SEB stimulation in type 2 CRSwNP. Notably, IL-4 stimulation led to an increase in oncostatin M (OSM) only in type 2 CRSwNP. Additionally, the number and proportion of TSLP- and OSM-expressing cells among CD163-positive cells were significantly higher in the NP of type 2 CRSwNP patients.

Conclusion. NP-derived macrophages from type 2 CRSwNP exhibit elevated expression of TSLP and OSM in response to IL-4 or SEB, potentially contributing to the pathophysiology of type 2 CRSwNP.

Keywords. *Rhinosinusitis; Nasal Polyps; Macrophages; Thymic Stromal Lymphopoietin; Oncostatin M*

INTRODUCTION

Chronic rhinosinusitis (CRS) is a heterogeneous disease that is phenotypically classified as chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP) [1]. Nasal polyps (NPs) are hyperplastic outgrowths of the inflamed sinonasal mucosa showing increased infiltration of diverse immune cells [2-5]. Its pathogenesis primarily involves an impaired epithelial barrier and a dysregulated immune response, leading to the failure of tissue homeostasis and inadequate protection against pathogens, allergens, and irritants [6,7]. This dis-

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ruption ultimately results in excessive tissue remodeling [5,6]. Tissue macrophages are essential for maintaining epithelial integrity and mucosal homeostasis, constituting the most abundant innate immune cells in NP [8,9]. They play a crucial role in protective immunity against external invasions and provide a rapid response to disruptions in the epithelial barrier [10]. Macrophages exhibit high plasticity and heterogeneity [11]. They can differentiate into proinflammatory M1 or anti-inflammatory M2 phenotypes, which contribute to Th1- and Th2-mediated forms of immune response [8,10,11]. Previous studies suggest that these macrophages may be involved in tissue remodeling [9,11-13]. Especially in NP, M2 macrophages are known for enhancing excessive fibrin deposition by releasing factor XIII-A [12]. Additionally, macrophages are also known as inducing epithelial mesenchymal transition (EMT) in fibrosis or cancer diseases [14-19]. An increased number of tissue macrophages producing matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) can contribute to edema, extracellular matrix degradation, and pseudocyst development, all of which exacerbate tissue remodeling in CRSwNP [9,11,13].

CRS endotypes are classified into type 2 and non-type 2 based on prevalent immune response that is related to etiology [1]. CRSwNP is more often associated with a type 2 inflammatory profile, which is of particular interest due to its poorer prognosis and higher recurrence [20-22]. Although M2 macrophages are significantly increased in NP compared to controls, their distribution across different CRS endotypes remains unclear [9].

Microenvironmental factors may further influence the inflammatory landscape of the sinonasal mucosa. For instance, lipopolysaccharide (LPS) contamination or an increased abundance of gram-negative bacteria in the airway microbiota exacerbates T helper 2 cell-type asthma [23-25]. Moreover, within the sinonasal tissue microenvironment of type 2 CRS has been shown to

induce steroid resistance, which may contribute to reduced therapeutic responsiveness [26]. IL-4 is a prototypical type 2 cytokine widely used to model type 2 inflammation *in vitro* [9,27]. Staphylococcal enterotoxin B (SEB), a key exotoxin secreted by *Staphylococcus aureus*, functions as a superantigen and has been implicated in the pathogenesis of type 2 CRS [28,29].

Despite these insights, the functional responses of NP-derived macrophages to distinct microenvironmental inflammatory stimuli—and their potential roles in CRSwNP pathogenesis—remain poorly understood. Therefore, we aimed to investigate the distribution and endotype-specific responses of NP-derived macrophages to key inflammatory cues including IL-4, SEB, and LPS, to better understand their contribution to the pathophysiology of CRSwNP.

MATERIALS AND METHODS

Study design and subjects

The study was conducted in accordance with the Helsinki Declaration and obtained approval from the Ethical Committee of Boramae Medical Center, Seoul, Korea (No. 20-2024-45 and 30-2020-160). Written informed consent was obtained from all participants prior to sample collection.

Patients with CRSwNP who underwent endoscopic sinus surgery were enrolled for NP collection. Patients were excluded if they were under 18 years of age, had unilateral rhinosinusitis, antrochoanal polyps, allergic fungal sinusitis, cystic fibrosis, immotile ciliary disease, or had received antibiotics, systemic or topical corticosteroids, or other immunomodulatory treatments within 4 weeks prior to surgery [28]. As controls, uncinate nasal mucosa tissues were obtained from individuals without sinonasal disease who were undergoing unrelated nasal surgeries, such as dacryocystorhinostomy, orbital decompression, or skull base surgery.

Type 2 CRSwNP in the bulk RNA sequencing and the *ex vivo* analysis were defined as absolute blood eosinophil counts ≥ 250 cells/ μ L from preoperative Complete Blood Count data [1]. For histological analysis, endotyping was defined by tissue eosinophil counts, as previously described [28,29]. The number of tissue eosinophils for endotyping was counted in the five densest visual fields ($\times 400$) by two independent observers (HC and DWK), and the mean values were calculated. Type 2 CRSwNP was defined as ≥ 55 tissue eosinophils (H&E) per high-power field (HPF) [30] in NP, non-type 2 CRSwNP was defined as < 55 tissue eosinophils/HPF [31].

Bulk RNA sequencing

Uncinate process mucosa from four control subjects and NP from 4 non-type 2 CRSwNP and 4 type 2 CRSwNP subjects were included in this study. Total RNA was isolated using the TRIzol reagent (Invitrogen). After the removal of contaminating

HIGHLIGHTS

- The proportion of M2 macrophages was higher in type 2 chronic rhinosinusitis with nasal polyps (CRSwNP), whereas both M1 and M2 macrophage populations were elevated in non-type 2 CRSwNP.
- Baseline cytokine analysis of NP-derived macrophages revealed elevated thymic stromal lymphopoietin (TSLP) levels in CRSwNP; moreover, TSLP expression was significantly upregulated following interleukin (IL)-4 or staphylococcal enterotoxin B stimulation in type 2 CRSwNP.
- The number and proportion of TSLP-expressing cells among CD163-positive cells were markedly higher in the NPs of type 2 CRSwNP patients.
- IL-4 stimulation induced oncostatin M (OSM) expression exclusively in type 2 CRSwNP; in parallel, the number and proportion of OSM-expressing cells among CD163-positive cells were significantly increased in the NPs.

DNA with DNase, libraries were prepared using the TruSeq Stranded Total RNA Library Prep Gold Kit (Illumina). The purified RNA was randomly fragmented and reverse-transcribed into complementary DNA (cDNA). Polymerase chain reaction amplification was performed, and GRCh38 was used as the reference genome. Inserts of 200–400 bp were selected, and paired-end sequencing was conducted. Preprocessing steps were undertaken to remove low-quality reads and artifacts. The preprocessed reads were mapped to the reference genome using HISAT2 (version 2.1.0), and aligned reads were generated with Bowtie2 (version 2.3.4.1). Transcript assembly was carried out using StringTie (version 2.1.3.b) based on the reference-aligned reads. Expression profiles were quantified as fragments per kilobase of transcript per million mapped reads, reads per kilobase of transcript per million mapped reads, and transcripts per kilobase million (TPM) values, which account for read count, transcript length, and sequencing depth. For immune cell fraction analysis, TPM-normalized data, with B mode batch correction, were analyzed using the CIBERSORTx program, with the LM22 signature matrix as a reference and 1,000 permutations [32].

Isolation of NP macrophages and measurement of released proteins

Nasal tissues from 16 patients were used for IL-4 and SEB stimulation analysis (4 controls, 6 non-type 2 CRSwNP, and 6 type 2 CRSwNP subjects). For LPS stimulation, samples from 23 patients were analyzed (2 controls, 8 non-type 2 CRSwNP, and 13 type 2 CRSwNP subjects). Single-cell isolation from NP tissues was performed using the OctoMACS separator kit (130-042-108, Miltenyi Biotec). NP tissues were placed in serum-free RPMI-1640 medium supplemented with collagenase (1 mg/mL) and DNase I (30 µg/mL). The NP tissues were washed twice with 1X phosphate-buffered saline (PBS), chopped into small pieces, and added to the MACS tube along with solution 1. Following the manufacturer's protocol, the tissue was processed twice, then incubated in a 37 °C CO₂ incubator for 30 minutes to 1 hour. Centrifugation was performed at 1,400 RPM for 5 minutes, the supernatant was discarded, and 5 mL of 1× red blood cell lysis buffer was added. After a 1-minute incubation at room temperature (RT), the sample was centrifuged again at 1,400 RPM for 3 minutes. The supernatant was discarded, and the cell pellets were resuspended in 2 mL of 0.5% RPMI-1640. CD14 microbeads (20 µL per 1×10⁷ cells) were added, mixed, and incubated at 4 °C for 15 minutes. A buffer solution was prepared by mixing 1× PBS, 2 mM ethylenediaminetetraacetic acid (EDTA), and 0.5% bovine serum albumin (BSA), followed by centrifugation at 4 °C, 300 g, for 10 minutes. After discarding the supernatant, 500 µL of buffer was added, and macrophages were separated using a magnetic separator. The isolated macrophages were divided into two or three groups: an untreated group, IL-4 (10 ng/mL) group, and SEB (1 ng/mL) group or untreated group and LPS (100 ng/mL) group [33,34]. Cells were

seeded into 24-well plates and incubated for 6 hours following treatment with IL-4 or SEB or LPS.

We selected four representative categories of cytokines and chemokines—type 2-related, neutrophil-associated, proinflammatory, and regulatory mediators—which were measured in the supernatants using a multiplex cytokine assay. These included type 2-related mediators such as thymic stromal lymphopoietin (TSLP), C-C motif chemokine ligand 24 (CCL24), bone morphogenetic protein 2 (BMP2) [35], and oncostatin M (OSM) [36]; neutrophil-associated mediators such as interleukin (IL)-17A, IL-8, granulocyte colony-stimulating factor (G-CSF), chemokine (C-X-C motif) ligand 1 (CXCL1), and MMP9; proinflammatory mediators including IL-6, tumor necrosis factor (TNF)-α, IL-1β, and VEGF; and regulatory cytokines such as IL-10 and transforming growth factor (TGF)-β. Baseline levels of cytokines and chemokines were normalized to relative cell counts (2×10⁵ cells), and the mean values were calculated and compared. MACS purity was assessed by FACS using a CD14 antibody (Miltenyi Biotec, CD14-PE, #130-110-519), which confirmed a purity of greater than 70%.

Immunofluorescence staining

Tissue sections were prepared following a previously described protocol [28]. NP were fixed in 4% paraformaldehyde, after which the tissues were dehydrated in ethyl alcohol, embedded in paraffin, and sectioned into 4 µm slices. The sections were deparaffinized, and antigen retrieval was performed. Permeabilization of the tissues was achieved by treating them with 0.3% Triton-X100 for 15 minutes at RT, followed by blocking with 5% BSA solution (Bovogen Biologicals Pty Ltd.) for 1 hour at RT. The slides were then incubated overnight at 4 °C with primary antibodies against CD163 (1:200, #ab156769, Abcam Inc.), TSLP (1:100, TA306385, OriGene), and OSM (1:50, bs-6295R, Bioss). After washing with 1× PBS, the sections were incubated with Cy3-conjugated immunoglobulin G (IgG) (1:1,000, #ab97035, Abcam; #ab6939, Abcam) and Alexa Fluor 488-conjugated IgG (1:1,000, #ab150113, Abcam; A-11008, Invitrogen). Nuclei were stained with Hoechst reagent (1:1,000, #H1399, Invitrogen). Each slide was analyzed using the CELENA S Digital Cell Imaging System (Logos Biosystems). Two independent observers assessed the average number of targeted cells in three representative fields exhibiting the greatest cellular infiltration. In cases of disagreement, a third independent observer was consulted to reach a consensus.

Statistical analysis

Continuous variables were presented as either the mean with standard deviation or the median with interquartile range, depending on the normality of the data. Normality was assessed using the Shapiro-Wilk test, as well as skewness and kurtosis. Comparisons between two groups were performed using either a two-tailed *t*-test or the Mann-Whitney *U*-test, while compari-

Table 1. Demographic characteristics

Variable	Control	Non-type 2 CRSwNP	Type 2 CRSwNP
Total no. of subjects	15	28	33
Age (yr)	62.00 (36.00–64.00)	54.00 (41.00–64.00)	52.00 (44.00–59.00)
Sex (male)	6 (40.00)	21 (75.00)	26 (75.79)
Blood eosinophil count (cells/ μ L)	137.34 (80.01–231.94)	159.84 (98.06–181.83)	421.74 (331.70–566.58)
Methodologies used			
Bulk RNA sequencing	4	4	4
<i>Ex vivo</i> and <i>in vitro</i>	6	14	19
Tissue IF	5	10	10

Values are presented as median (interquartile range) or number (%).
CRSwNP, chronic rhinosinusitis with nasal polyps; IF, immunofluorescence.

sons involving three or more groups were conducted using analysis of variance or the Kruskal-Wallis test, with the selection of tests dependent on the normality. To adjust for age and sex, we initially performed multivariate linear regression. When the residuals violated the assumption of normality, a log($x+1$) transformation was applied to the dependent variable. If the residuals continued to deviate from normality despite the transformation, we employed robust rank-based regression to ensure valid inference. For multiple comparisons, Dunn's test was employed. Paired samples were analyzed using the two-tailed t or Wilcoxon signed-rank test. Categorical variables were analyzed using the chi-square test. All statistical analyses were carried out using R for Windows, version 4.0.3 (R Foundation for Statistical Computing). A P -value of less than 0.05 was considered statistically significant.

RESULTS

Tissue macrophage subsets in endotypes of CRSwNP

Demographic data for each study is shown in Table 1 (Supplementary Tables 1–4 for details). We utilized CIBERSORT to assess the fractions of 22 immune cell types in type 2 and non-type 2 NP. Compared to controls, the fraction of M2 macrophages was significantly increased in type 2 CRSwNP. Although both M1 and M2 macrophage fractions were elevated in non-type 2 CRS, these increases did not reach statistical significance (Fig. 1). After adjusting for age and sex using a robust rank-based regression model, the type 2 group demonstrated a significant increase in M2 macrophages (estimate=0.08, standard error=0.02, $t=3.40$, $P=0.011$), while the increase in M1 macrophages was not statistically significant (robust rank-based regression, estimate=−0.01, standard error=0.01, $t=−0.40$, $P=0.699$). In contrast, the non-type 2 group showed statistically significant increases in both M1 (robust rank-based regression, estimate=0.03, standard error=0.01, $t=2.69$, $P=0.031$) and M2 macrophages (estimate=0.06, standard error=0.02, $t=2.78$, $P=0.027$) compared to controls.

Baseline cytokine and chemokine profiles of NP-derived macrophages

Baseline cytokine and chemokine levels released from untreated NP-derived macrophages were analyzed and compared across different endotypes (Fig. 2). Among these, higher levels of TSLP were released in CRSwNP regardless of endotype, compared to controls. CCL24/Eotaxin-2 was produced more from the macrophage of type 2 CRSwNP than controls.

After adjusting for age and sex, baseline TSLP levels were increased in both the type 2 (estimate=1.70, standard error=0.82, $t=2.08$, $P=0.076$) and non-type 2 (estimate=1.22, standard error=0.86, $t=1.41$, $P=0.199$) groups, although these increases did not reach statistical significance. CCL24/Eotaxin-2 was significantly elevated in the type 2 group (log-transformed; estimate=2.51, standard error=0.88, $t=2.86$, $P=0.024$). Additionally, MMP9 levels were significantly increased in both type 2 (log-transformed; estimate=2.21, standard error=0.91, $t=2.42$, $P=0.022$) and non-type 2 (log-transformed; estimate=3.01, standard error=0.91, $t=3.30$, $P=0.003$) groups. In the non-type 2 group, IL-8/CXCL8 levels were significantly elevated (log-transformed; estimate=1.41, standard error=0.52, $t=2.71$, $P=0.011$), while the type 2 group exhibited a non-significant trend towards increased IL-8/CXCL8 secretion (log-transformed; estimate=0.91, standard error=0.52, $t=1.76$, $P=0.089$). IL-1 β showed a trend toward elevation in the type 2 group (log-transformed; estimate=1.72, standard error=0.93, $t=1.86$, $P=0.073$), whereas IL-6 secretion was significantly increased only in the non-type 2 group (log-transformed; estimate=1.33, standard error=0.62, $t=2.15$, $P=0.039$). TNF- α levels were also significantly elevated in the non-type 2 group (log-transformed; estimate=1.43, standard error=0.64, $t=2.22$, $P=0.034$) (Table 2).

NP-derived macrophages response to IL-4, SEB, and LPS

Cytokine and chemokine secretion levels were compared in each sample by evaluating untreated and treated groups to assess their responses to various external stimuli using a paired test (Supplementary Fig. 1). The response of NP-derived macrophages to IL-4 challenge resulted in elevated levels of type 2-associated (TSLP and BMP2), regulatory cytokines (IL-10), and CXCL1 in

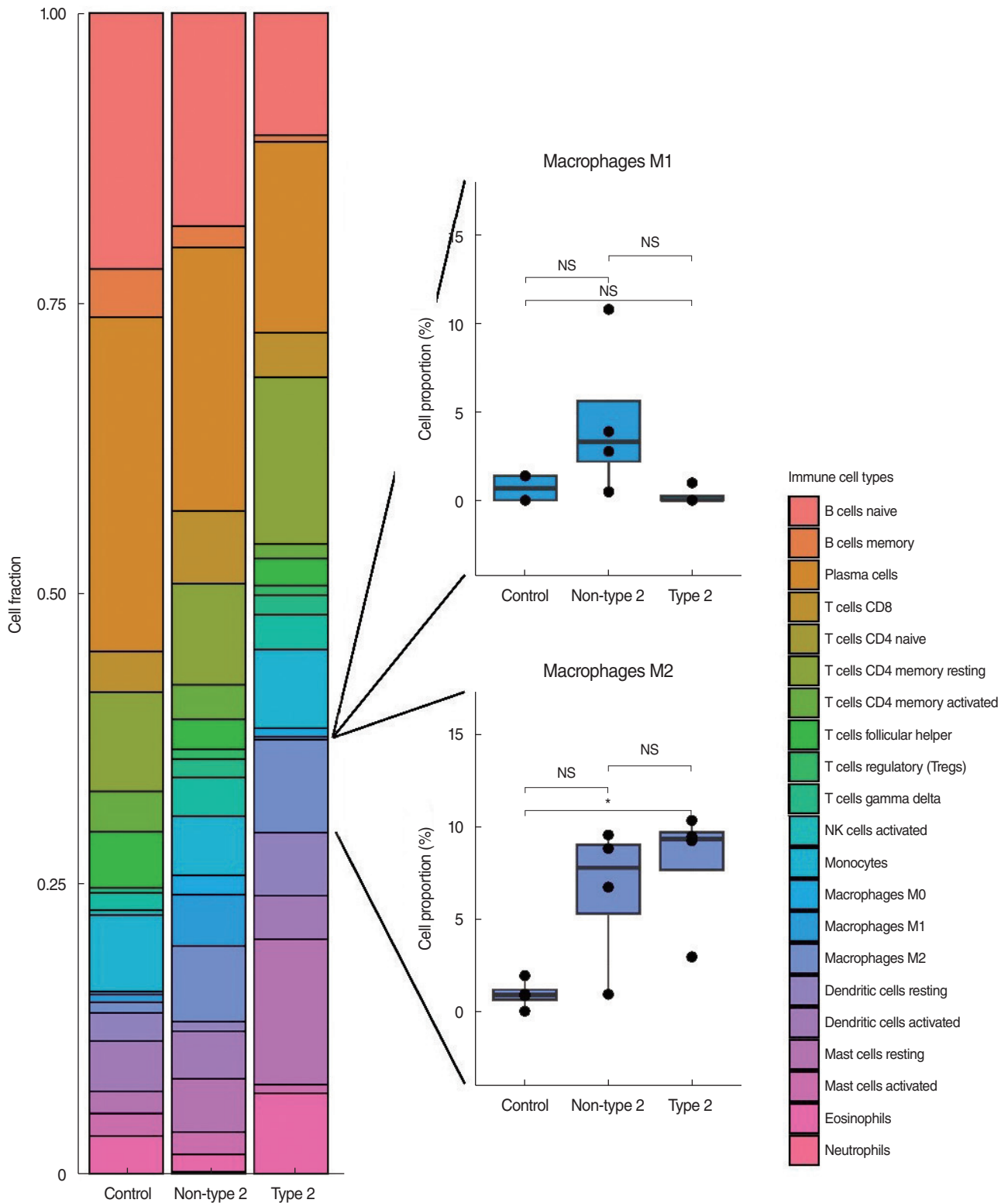


Fig. 1. Proportions of M1 and M2 macrophages in nasal polyps. The proportion of M2 macrophages is significantly elevated in type 2 chronic rhinosinusitis with nasal polyps compared to controls. Control: n=4; Non-type 2: n=4; Type 2: n=4. Statistical significance is indicated as follows: NS (not significant) denotes $P>0.05$, $*P<0.05$.

both endotypes (Fig. 3). OSM levels showed a significant increase in type 2 CRSwNP, whereas T3 cytokine such as IL-17A levels decreased (Fig. 3).

NP-derived macrophage responses to SEB challenge resulted in elevated levels of BMP2 in both endotypes of CRSwNP and TSLP in type 2 CRSwNP (Fig. 3). In non-type 2 CRSwNP, significant increases were observed in CXCL1. Additionally, IL-6 levels were reduced in non-type 2 CRSwNP. The response of

NP-derived macrophages to LPS challenge showed a consistent pattern regardless of endotype (Fig. 3): OSM, type 2 related mediator, proinflammatory cytokines such as TNF- α , IL-1 β , IL-6, and VEGF; neutrophils-associated mediators such as IL-8/CXCL8 and CXCL1; regulatory cytokines such as IL-10 and TGF- β were increased with LPS challenge. Intriguingly, MMP9 was exclusively produced more in response to LPS in type 2 NP-derived macrophage.

	Control	Non-type 2	Type 2	
Type 2 related	3.1	10.8	19.2	TSLP
	0.0	11.0	155.9	CCL24/Eotaxin-2
	302.9	303.0	288.0	BMP2
	2,357.2	2,799.3	2,901.9	OSM
Neutrophils	18.1	14.3	28.9	IL-17A
	2,769.2	8,170.1	5,962.4	IL-8/CXCL8
	0.0	5.3	19.6	G-CSF
	6,824.6	14,104.1	7,035.0	MMP9
	1,362.6	882.4	997.0	CXCL1
Proinflammatory	144.5	156.1	238.7	IL-6
	16.3	118.4	29.1	TNF- α
	4.5	159.4	112.4	IL-1 β
	4.5	1.7	5.8	VEGF
Regulatory	10.6	14.7	10.1	IL-10
	5,294.3	691.8	806.8	TGF- β

Concentration significantly higher than 2 other groups

Concentration significantly higher than another group

Fig. 2. Baseline cytokine levels secreted by macrophages from nasal polyp. The cytokine secretion levels, normalized to a relative cell count of 2×10^5 cells, are expressed in units of pg/mL. TSLP, thymic stromal lymphopoietin; CCL24, C-C motif chemokine ligand 24; BMP2, bone morphogenetic protein 2; OSM, oncostatin M; IL, interleukin; CXCL, chemokine (C-X-C motif) ligand; G-CSF, granulocyte colony-stimulating factor; MMP9, matrix metalloproteinase 9; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; TGF, transforming growth factor. Kruskal-Wallis test, control: n=6; Non-type 2: n=14; Type 2: n=19.

Expression of TSLP and OSM in the macrophages of type 2 CRSwNP

Notably, our findings demonstrated that baseline TSLP secretion levels were higher in macrophages derived from NP compared to controls (Fig. 2). Furthermore, TSLP secretion increased in NP-derived macrophages following IL-4 stimulation; SEB stimulation induced TSLP in type 2 CRSwNP; Moreover, IL-4 stimulation specifically enhanced OSM secretion in type 2 NP-derived macrophages (Fig. 3). To validate these findings in NP tissues, immunofluorescence (IF) staining was performed on a total of 25 samples (5 controls, 10 non-type 2 NP, and 10 type 2 NP) and the distribution of CD163⁺ cells expressing TSLP and OSM was determined. In our examination of M2 macrophages, CD163 served as a key marker for this phenotype [37,38]. The number of TSLP-expressing CD163⁺ cells was significantly higher in patients with type 2 CRSwNP, compared to both controls and non-type 2 CRSwNP (Fig. 4A and B). This difference remained statistically significant in type 2 CRSwNP after adjusting for age and sex using multivariate linear regression (estimate=12.04, standard error=3.82, $t=3.15$, $P=0.005$). Additionally, the proportion of TSLP-expressing cells among CD163⁺ cells was higher in type 2 CRSwNP compared to controls (Fig. 4C), and this increase also remained statistically significant after adjustment for age and sex (estimate=0.28, standard error=0.13, $t=2.23$, $P=0.038$). The number of OSM-expressing CD163⁺ cells was also elevated in type 2 CRSwNP, both in absolute count and in proportion, compared to controls ($P=0.029$) (Fig. 5). After adjusting for age and sex, the type 2 endotype showed a statistically sig-

Table 2. Age- and sex-adjusted levels of representative cytokines estimated by multivariable linear regression (log-transformed)

Cytokine	Endotype	Estimate	Standard error	<i>t</i>	<i>P</i> -value
TSLP	Type 2	1.70	0.82	2.08	0.076
	Non-type 2	1.22	0.86	1.41	0.199
CCL24/Eotaxin-2	Type 2	2.51	0.88	2.86	0.024
	Non-type 2	2.21	0.91	2.42	0.022
MMP9	Type 2	3.01	0.91	3.30	0.003
	Non-type 2	0.91	0.52	1.76	0.089
IL-8/CXCL8	Type 2	1.41	0.52	2.71	0.011
	Non-type 2	1.72	0.93	1.86	0.073
IL-1 β	Type 2	1.33	0.62	2.15	0.039
	Non-type 2	1.43	0.64	2.22	0.034

TSLP, thymic stromal lymphopoietin; CCL24, C-C motif chemokine ligand 24; MMP9, matrix metalloproteinase 9; IL, interleukin; CXCL8, chemokine (C-X-C motif) ligand 8; TNF, tumor necrosis factor.

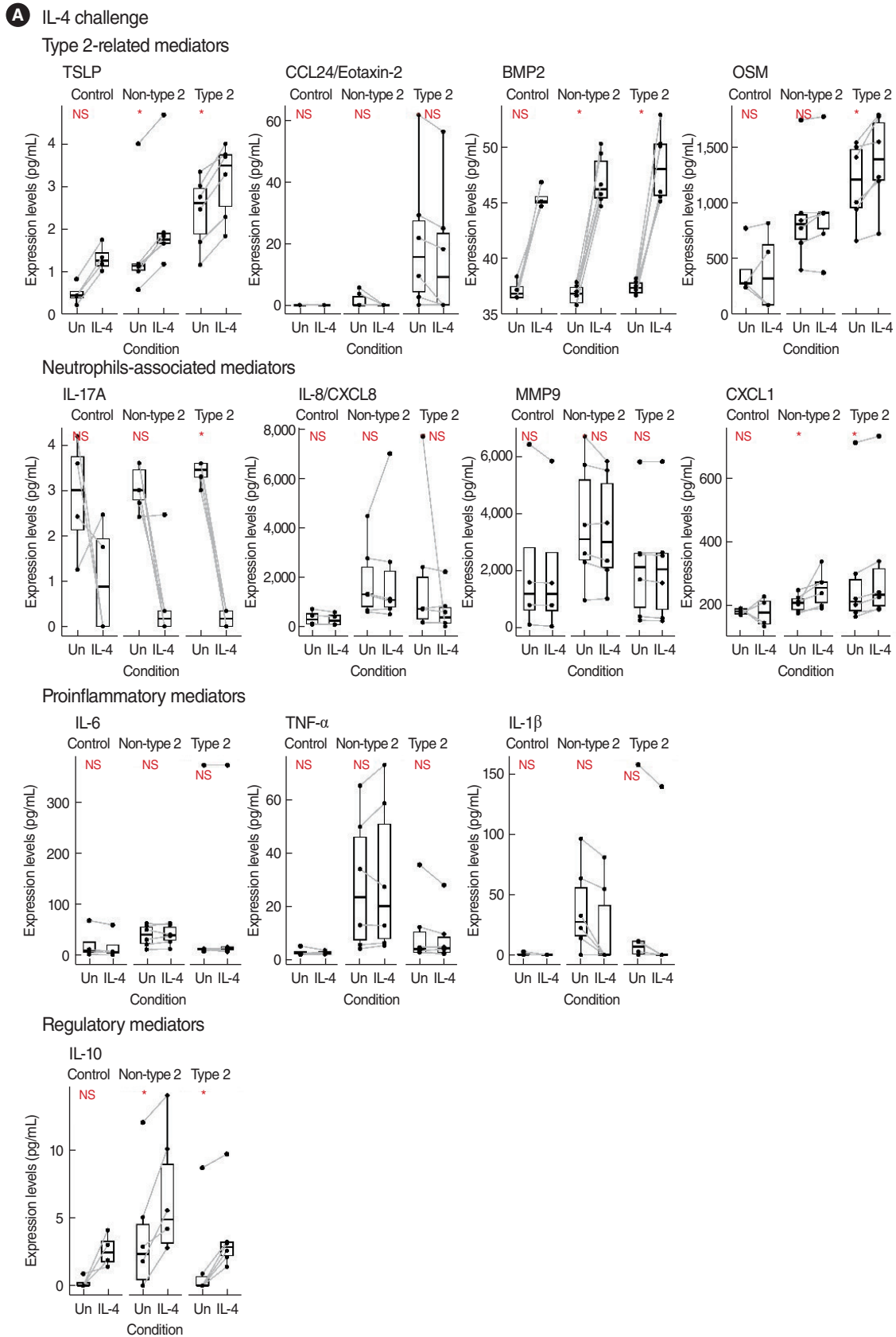
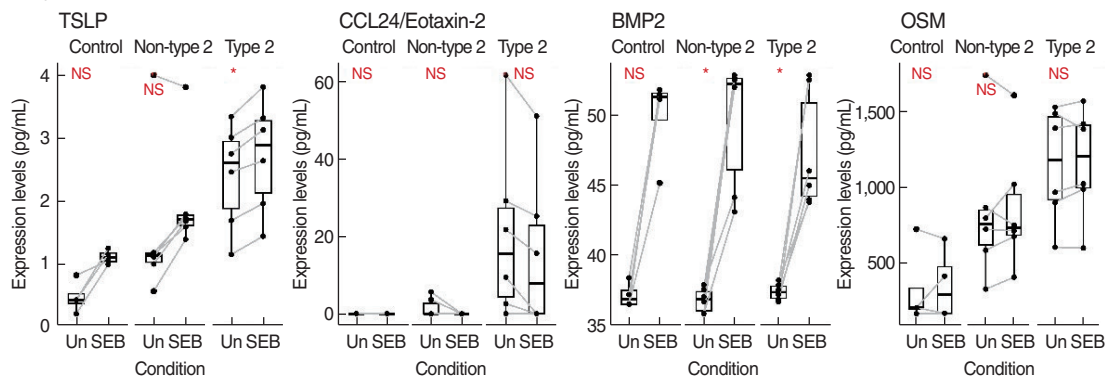


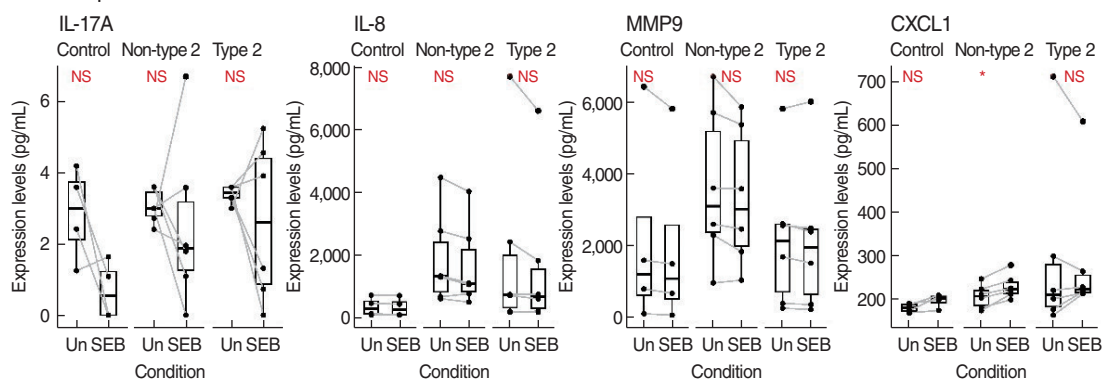
Fig. 3. Continued

B SEB challenge

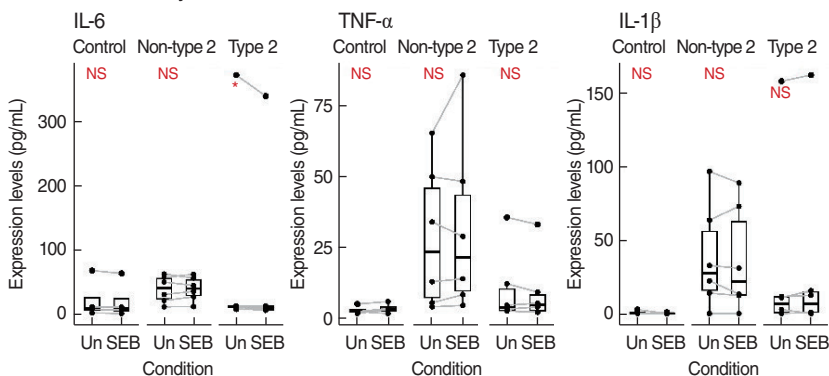
Type 2-related mediators



Neutrophils-associated mediators



Proinflammatory mediators



Regulatory mediators

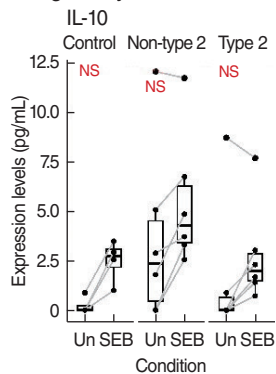


Fig. 3. Continued

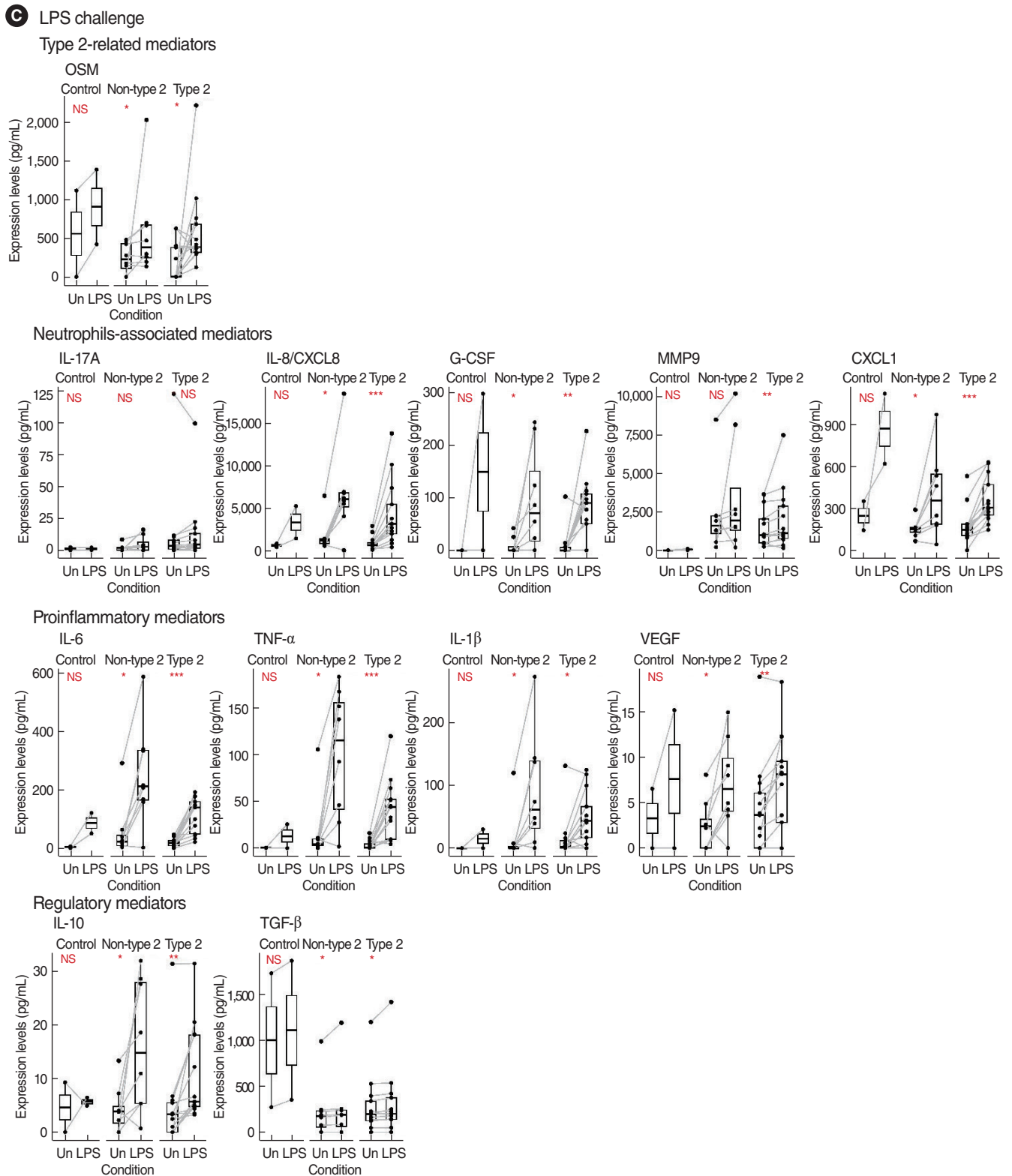


Fig. 3. Cytokine levels secreted by macrophages in response to (A) interleukin (IL)-4, (B) staphylococcal enterotoxin B (SEB) (control: $n=4$; Non-type 2: $n=6$; Type 2: $n=6$), and (C) lipopolysaccharide (LPS) (control: $n=2$; Non-type 2: $n=8$; Type 2: $n=13$) stimulation. Statistical analysis was performed using a paired test. TSLP, thymic stromal lymphopoietin; CCL24, C-C motif chemokine ligand 24; BMP2, bone morphogenetic protein 2; OSM, oncostatin M; CXCL, chemokine (C-X-C motif) ligand; MMP9, matrix metalloproteinase 9; TNF, tumor necrosis factor. NS (not significant) denotes $P>0.05$, $*P<0.05$, $**P<0.01$, $***P<0.001$.

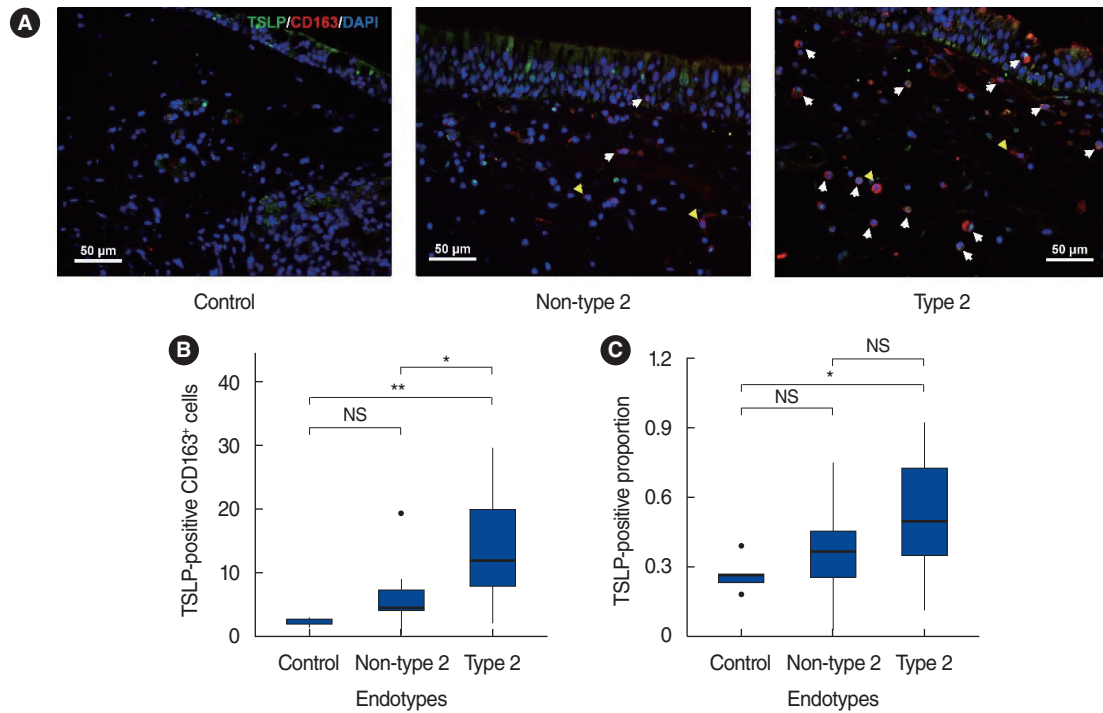


Fig. 4. Expression of thymic stromal lymphopoietin (TSLP) on CD163⁺ cells. The white arrows indicate TSLP-positive CD163⁺ cells, while the yellow arrowheads indicate TSLP-negative CD163⁺ cells. (A) Immunofluorescence study showing CD163⁺ cells with TSLP expression across different endotypes. (B) Counts of TSLP-positive CD163⁺ cells by endotype. (C) Proportions of TSLP-positive CD163⁺ cells by endotype. Control, n=5; Non-type 2, n=10; Type 2, n=10. NS (not significant) denotes $P>0.05$, * $P<0.05$, ** $P<0.01$.

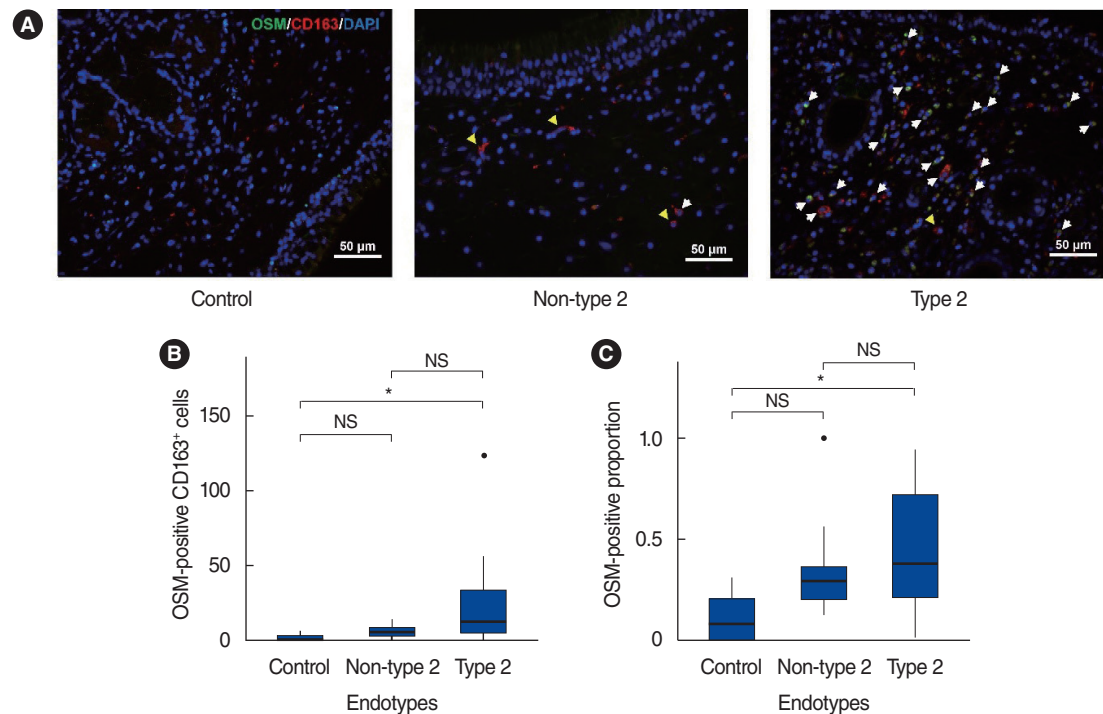


Fig. 5. Expression of oncostatin M (OSM) on CD163⁺ cells. The white arrows indicate OSM-positive CD163⁺ cells, while the yellow arrowheads indicate OSM-negative CD163⁺ cells. (A) Immunofluorescence study showing CD163⁺ cells with OSM expression across different endotypes. (B) Counts of OSM-positive CD163⁺ cells by endotype. (C) Proportions of OSM-positive CD163⁺ cells by endotype. Control, n=5; Non-type 2, n=10; Type 2, n=10. NS (not significant) denotes $P>0.05$, * $P<0.05$.

nificant increase in both OSM-expressing cell count (log transformed, estimate=1.86, standard error=0.63, $t=2.98$, $P=0.007$) and proportion (estimate=0.34, standard error=0.16, $t=2.19$, $P=0.041$).

DISCUSSION

In this study, we investigated the distribution of macrophages and their cytokine expression across different endotypes of CRSwNP, as well as their responses to external stimuli, including IL-4, SEB, and LPS. Regarding the distribution of macrophages across different endotypes of CRSwNP, a previous study has reported an elevated proportion of M2 macrophages in NP compared to healthy controls, while another study suggested that differentiation of M1 macrophages is related to non-type 2 airway inflammation [9,39]. However, how the distribution of M1 and M2 macrophages differs according to CRSwNP endotypes remains unknown. Notably, our findings revealed a higher proportion of M2 macrophages within the immune cell population in type 2 CRSwNP compared to healthy controls. Furthermore, both M1 and M2 macrophages were increased in non-type 2 CRS. In brief, although the difference was not statistically significant in non-type 2, M2 macrophages are higher in both endotypes of CRSwNP compared to controls. This finding aligns with a previous study suggesting a key role of M2 macrophages in the pathogenesis of CRSwNP [8,9].

We assessed both the baseline cytokine levels of macrophages in CRSwNP and their responses to external stimuli such as IL-4, SEB, and LPS. IL-4 is a representative type 2 immune stimulant [9,27]. SEB, a key toxin secreted by *S. aureus*, acts as a superantigen involved in the pathogenesis of type 2 CRS [40,41]. We assessed the expression levels of representative type 2-related mediators, including TSLP, CCL24, BMP2, and OSM; neutrophil-associated mediators, such as IL-17A, IL-8, G-CSF, CXCL1, and MMP9; proinflammatory mediators, including IL-6, TNF- α , IL-1 β , and VEGF; as well as regulatory cytokines, such as IL-10 and TGF- β . Interestingly, after stimulation with IL-4, SEB, and LPS, the cytokine profiles of the macrophages overlapped and their responses were broadly consistent, regardless of the endotype. These findings suggest that although tissue macrophages are already primed and differentiated, they remain similarly responsive to strong external stimuli such as IL-4, SEB, and LPS, highlighting the importance of environmental factors in disease control. However, certain cytokines and chemokines exhibited distinct differences depending on the endotype, which are of particular importance in our research.

In our study, baseline cytokine levels in macrophages isolated *ex vivo* from CRSwNP indicated upregulation of TSLP compared to healthy controls. The type 2-related stimuli, IL-4 and SEB further increased the secretion of TSLP in macrophages from type 2 CRSwNP. This response was also confirmed in human NP tis-

sue using IF staining. In the IF study, we identified significant expression of TSLP in CD163⁺ cells derived from type 2 CRSwNP both in counts and proportions. TSLP is primarily recognized as an epithelial cell-derived cytokine that initiates type 2 immune responses and promotes tissue eosinophilia [36,42-47]. Under chronic inflammatory conditions, TSLP can also induce goblet cell hyperplasia and excessive mucus production [48]. It has also been implicated in tissue fibrosis, contributing to airway thickening and wall remodeling [49,50]. Notably, our findings indicate that CD163⁺ macrophages—likely M2 macrophages—represent an additional source of TSLP in CRSwNP, suggesting that it may not only drive type 2 immune responses but also act as a secondary response to ongoing inflammation, potentially influencing disease severity and symptom control through mucus hypersecretion and tissue fibrosis.

Another cytokine that drew our attention was OSM. It is a type 2-related cytokine associated with poor outcomes in CRS, as it enhances TSLP expression through IL-4 signaling and may contribute to airway barrier dysfunction [46,51,52]. Beyond its immunological role, OSM is implicated in both ossification and EMT [53-55]. It promotes bone formation by inhibiting M1 polarization and promoting M2 polarization [53]. Furthermore, macrophages isolated from patients with neurogenic heterotopic ossification overproduce OSM in response to LPS stimulation [54]. Also, macrophage-derived OSM induced by LPS activates inflammatory and mucus-producing pathways [56]. In breast cancer, OSM promotes EMT, thereby indirectly driving disease progression [55]. In our study, LPS stimulation induced a significant increase in OSM expression in CRSwNP-derived macrophages, irrespective of endotype, while baseline OSM secretion remained stable. By contrast, IL-4 stimulation specifically increased OSM expression in type 2 CRSwNP macrophages. Collectively, these findings suggest that macrophages may contribute to mucus production, ossification, EMT, and disease progression in CRSwNP through OSM production.

Although macrophages have been reported to produce TSLP and OSM, these data extend prior epithelial-focused reports by identifying macrophage-derived TSLP and OSM as endotype-biased amplifiers of established type-2 cytokine pathways [54,57]. While the functional impact of macrophage-derived TSLP and OSM on clinical outcomes and disease control has not yet been clearly established, these observations suggest a macrophage-mediated amplification loop that may potentiate type-2 inflammation in type 2 CRSwNP. Our findings therefore warrant further investigation into their clinical relevance. Interestingly, while baseline cytokine measurements of *ex vivo* macrophages showed a numerical increase in OSM secretion in the type 2 CRSwNP group, the difference was not statistically significant compared to other groups. In contrast, IF revealed a significantly higher number and proportion of OSM-expressing macrophages in type 2 NPs compared to controls. This discrepancy may be due to the limited sample size, or it may reflect methodological

differences—whereas immunohistochemistry assesses intracellular protein expression, *ex vivo* cytokine measurements quantify secreted protein levels. Therefore, these findings should be interpreted with caution.

Next, our data showed an increased BMP2 expression in type 2 CRSwNP macrophages following stimulation with IL-4 and SEB. In our previous study, we already demonstrated that BMP2 concentrations were elevated in patients with neo-osteogenesis and more extensive disease, with eosinophils and macrophages identified as key sources [35]. M1 macrophages produce pro-inflammatory cytokines, while M2 macrophages are recognized for promoting anti-inflammatory responses [9,27]. As previously mentioned, macrophages are also known to be involved in epithelial mesenchymal transition [14-19]. Additionally, they are suggested to be involved in CRSwNP pathogenesis by facilitating tissue remodeling by increased MMP9, TGF- β , VEGF, and PDGF [9,58]. Consistent with previous studies, our study data also showed TGF- β and VEGF were increased in CRSwNP after LPS stimulation. They play a defensive role against external invaders, but they are also associated with tissue damage. The fact that they significantly increase only in CRSwNP under the same stimuli, suggesting a link to disease progression, is an interesting finding that warrants further investigation.

Our study has several limitations. First, although CIBERSORTx enables detailed immune cell profiling based on a broad reference gene set, its estimations cannot fully substitute for direct validation [32,59]. Second, the relatively small sample size reduces the robustness of conclusions. Third, while CD163 is a well-established marker for M2 macrophages, additional markers and functional validation across larger cohorts would provide greater accuracy in macrophage characterization [37,38]. Another limitation of this study is that different criteria were used to define type 2 CRS in the *ex vivo* analyses (including RNA-seq) and in the histological analysis; therefore, direct comparison between these datasets should be interpreted with caution. Furthermore, given the 70.4% purity of macrophages isolated *in vitro*, the influence of contaminating non-macrophage cells cannot be completely ruled out. Lastly, our study lacks long-term clinical outcome data. While this limits the ability to directly correlate our findings with disease progression or recurrence, type 2 CRSwNP is already well established as being associated with higher recurrence rates and poorer clinical outcomes. Accordingly, we focused our analysis on macrophages derived from type 2 CRSwNP tissue. In addition, we did not conduct mechanistic experiments to determine how macrophage-derived OSM or TSLP contribute to pathogenesis; therefore, further studies are needed to elucidate the underlying pathways.

In conclusion, the proportion of M2 macrophages is elevated in type 2 CRSwNP; type 2 NP-derived M2 macrophages show higher expression of TSLP and OSM under type 2 microenvironment—cytokines that are known to play important roles in the pathophysiology of type 2 CRSwNP.

CONFLICT OF INTEREST

Dae Woo Kim is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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AUTHOR CONTRIBUTIONS

Conceptualization: DWK, AJ. Methodology: DWK, AJ, HSL, KME. Formal analysis: HC, AJ. Data curation: HC, DWK. Visualization: HC. Project administration: KME, JAP. Funding acquisition: HC, DWK. Writing—original draft: HC, DWK. Writing—review & editing: HC, DWK, AJ, HSL, KME, JAP, SNH. All authors read and agreed to the published version of the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found online at <https://doi.org/10.21053/ceo.2025-00142>.

REFERENCES

1. Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al. European position paper on rhinosinusitis and nasal polyps 2020. *Rhinology*. 2020 Feb;58(Suppl S29):1-464.
2. Shah SA, Kobayashi M. Pathogenesis of chronic rhinosinusitis with nasal polyp and a prominent T2 endotype. *Heliyon*. 2023 Sep;9(9):e19249.
3. Newton JR, Ah-See KW. A review of nasal polyposis. *Ther Clin Risk*

- Manag. 2008 Apr;4(2):507-12.
4. delToro E, Hardin FML, Portela J. Nasal polyps [Internet]. StatPearls Publishing; 2025 [cited 2025 Dec 5]. Available from: <https://pubmed.ncbi.nlm.nih.gov/32809581/>
5. Liu K, Xu Y. Downregulation of TET2 contributes to nasal polypogenesis through hypoxia-inducible factor 1 α -mediated epithelial-to-mesenchymal transition. *Clin Exp Otorhinolaryngol*. 2024 Feb;17(1):64-77.
6. Huang ZQ, Liu J, Sun LY, Ong HH, Ye J, Xu Y, et al. Updated epithelial barrier dysfunction in chronic rhinosinusitis: targeting pathophysiology and treatment response of tight junctions. *Allergy*. 2024 May;79(5):1146-65.
7. Zhang M, Peng X, Liang X, Wang W, Yang Y, Xu F, et al. MicroRNA-145-5p regulates the epithelial-mesenchymal transition in nasal polyps by targeting Smad3. *Clin Exp Otorhinolaryngol*. 2024 May;17(2):122-36.
8. Banks CA, Schlosser RJ, Wang EW, Casey SE, Mulligan RM, Mulligan JK, et al. Macrophage infiltrate is elevated in CRSwNP sinonasal tissue regardless of atopic status. *Otolaryngol Head Neck Surg*. 2014 Aug;151(2):215-20.
9. Zhu Y, Sun X, Tan S, Luo C, Zhou J, Zhang S, et al. M2 macrophage-related gene signature in chronic rhinosinusitis with nasal polyps. *Front Immunol*. 2022 Nov;13:1047930.
10. Mowat AM, Bain CC. Mucosal macrophages in intestinal homeostasis and inflammation. *J Innate Immun*. 2011 Sep;3(6):550-64.
11. Fan HL, Han ZT, Gong XR, Wu YQ, Fu YJ, Zhu TM, et al. Macrophages in CRSwNP: do they deserve more attention? *Int Immunopharmacol*. 2024 Jun;134:112236.
12. Takabayashi T, Kato A, Peters AT, Hulse KE, Suh LA, Carter R, et al. Increased expression of factor XIII-A in patients with chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2013 Sep;132(3):584-92.e4.
13. Shi LL, Ma J, Deng YK, Chen CL, Wang H, Cao PP, et al. Cold-inducible RNA-binding protein contributes to tissue remodeling in chronic rhinosinusitis with nasal polyps. *Allergy*. 2021 Feb;76(2):497-509.
14. Tekin C, Abernethy HL, Waasdorp C, Hooijer GK, de Boer OJ, Dijk F, et al. Macrophage-secreted MMP9 induces mesenchymal transition in pancreatic cancer cells via PAR1 activation. *Cell Oncol (Dordr)*. 2020 Dec;43(6):1161-74.
15. Liu CY, Xu JY, Shi XY, Huang W, Ruan TY, Xie P, et al. M2-polarized tumor-associated macrophages promoted epithelial-mesenchymal transition in pancreatic cancer cells, partially through TLR4/IL-10 signaling pathway. *Lab Invest*. 2013 Jul;93(7):844-54.
16. Yu CC, Chien CT, Chang TC. M2 macrophage polarization modulates epithelial-mesenchymal transition in cisplatin-induced tubulointerstitial fibrosis. *Biomedicine (Taipei)*. 2016 Mar;6(1):5.
17. Anthony B, Allen JT, Li YS, McManus DP. Hepatic stellate cells and parasite-induced liver fibrosis. *Parasit Vectors*. 2010 Jul;3(1):60.
18. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol*. 2002 May;3(5):349-63.
19. Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest*. 2007 Mar;117(3):524-9.
20. Nakayama T, Sugimoto N, Okada N, Tsurumoto T, Mitsuyoshi R, Takaiishi S, et al. Jesrec score and mucosal eosinophilia can predict endotypes of chronic rhinosinusitis with nasal polyps. *Auris Nasus Larynx*. 2019 Jun;46(3):374-83.
21. Tokunaga T, Sakashita M, Haruna T, Asaka D, Takeno S, Ikeda H, et al. Novel scoring system and algorithm for classifying chronic rhinosinusitis: the JESREC study. *Allergy*. 2015 Aug;70(8):995-1003.
22. Yang SK, Kim JW, Won TB, Rhee CS, Han YB, Cho SW, et al. Differences in clinical and immunological characteristics according to the various criteria for tissue eosinophilia in chronic rhinosinusitis with nasal polyps. *Clin Exp Otorhinolaryngol*. 2023 Nov;16(4):359-68.
23. Czimmerer Z, Halasz L, Daniel B, Varga Z, Bene K, Domokos A, et al. The epigenetic state of IL-4-polarized macrophages enables inflammatory cytokine expansion and extended synergistic response to TLR ligands. *Immunity*. 2022 Nov;55(11):2006-26.e6.
24. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K, et al. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med*. 2002 Dec;196(12):1645-51.
25. Huang YJ, Nelson CE, Brodie EL, Desantis TZ, Baek MS, Liu J, et al. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *J Allergy Clin Immunol*. 2011 Feb;127(2):372-81.e1-3.
26. Wang SB, Chen SM, Zhu KS, Zhou B, Chen L, Zou XY, et al. Increased lipopolysaccharide content is positively correlated with glucocorticoid receptor-beta expression in chronic rhinosinusitis with nasal polyps. *Immun Inflamm Dis*. 2020 Dec;8(4):605-14.
27. Ishida K, Nagatake T, Saika A, Kawai S, Node E, Hosomi K, et al. Induction of unique macrophage subset by simultaneous stimulation with LPS and IL-4. *Front Immunol*. 2023 Apr;14:1111729.
28. Cha H, Lim HS, Park JA, Jo A, Ryu HT, Kim DW, et al. Effects of neutrophil and eosinophil extracellular trap formation on refractoriness in chronic rhinosinusitis with nasal polyps. *Allergy Asthma Immunol Res*. 2023 Jan;15(1):94-108.
29. Kim DK, Kim JY, Han YE, Kim JK, Lim HS, Eun KM, et al. Elastase-positive neutrophils are associated with refractoriness of chronic rhinosinusitis with nasal polyps in an Asian population. *Allergy Asthma Immunol Res*. 2020 Jan;12(1):42-55.
30. Toro MDC, Antonio MA, Alves Dos Reis MG, de Assumpcao MS, Sakano E. Achieving the best method to classify eosinophilic chronic rhinosinusitis: a systematic review. *Rhinology*. 2021 Aug;59(4):330-9.
31. Ikeda K, Shiozawa A, Ono N, Kusunoki T, Hirotsu M, Homma H, et al. Subclassification of chronic rhinosinusitis with nasal polyp based on eosinophil and neutrophil. *Laryngoscope*. 2013 Nov;123(11):E1-9.
32. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods*. 2015 May;12(5):453-7.
33. Haimel P, Bernhardt U, Schindela S, Henkel FD, Lechner A, Zissler UM, et al. Inflammatory macrophage memory in nonsteroidal anti-inflammatory drug-exacerbated respiratory disease. *J Allergy Clin Immunol*. 2021 Feb;147(2):587-99.
34. Kwon DH, Cha HJ, Choi EO, Leem SH, Kim GY, Moon SK, et al. Schisandrin A suppresses lipopolysaccharide-induced inflammation and oxidative stress in RAW 264.7 macrophages by suppressing the NF- κ B, MAPKs and PI3K/Akt pathways and activating Nrf2/HO-1 signaling. *Int J Mol Med*. 2018 Jan;41(1):264-74.
35. Kim JY, Lim S, Lim HS, Kim YS, Eun KM, Khalmuratova R, et al. Bone morphogenetic protein-2 as a novel biomarker for refractory chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2021 Aug;148(2):461-72.e13.
36. Kong IG, Kim DW. Pathogenesis of recalcitrant chronic rhinosinusitis: the emerging role of innate immune cells. *Immune Netw*. 2018 Apr;18(2):e6.
37. Hu JM, Liu K, Liu JH, Jiang XL, Wang XL, Chen YZ, et al. CD163 as a marker of M2 macrophage, contribute to predict aggressiveness and prognosis of Kazakh esophageal squamous cell carcinoma. *Oncotarget*. 2017 Mar;8(13):21526-38.
38. Tremble LF, McCabe M, Walker SP, McCarthy S, Tynan RF, Beecher S, et al. Differential association of CD68+ and CD163+ macrophages with macrophage enzymes, whole tumour gene expression and overall survival in advanced melanoma. *Br J Cancer*. 2020 Nov;123(10):1553-61.
39. Kim SR. Next-generation therapeutic approaches for uncontrolled asthma: insights into the heterogeneity of non-type 2 inflammation.

- Allergy Asthma Immunol Res. 2024 Jan;16(1):1-5.
40. Rouyar A, Classe M, Gorski R, Bock MD, Le-Guern J, Roche S, et al. Type 2/Th2-driven inflammation impairs olfactory sensory neurogenesis in mouse chronic rhinosinusitis model. *Allergy*. 2019 Mar;74(3):549-59.
41. Chegini Z, Didehdar M, Khoshbayan A, Karami J, Yousefimashef M, Shariati A, et al. The role of Staphylococcus aureus enterotoxin B in chronic rhinosinusitis with nasal polypsis. *Cell Commun Signal*. 2022 Mar;20(1):29.
42. Takai T. Tslp expression: cellular sources, triggers, and regulatory mechanisms. *Allergol Int*. 2012 Mar;61(1):3-17.
43. Kato A. Immunopathology of chronic rhinosinusitis. *Allergol Int*. 2015 Apr;64(2):121-30.
44. Khalmuratova R, Park JW, Shin HW. Immune cell responses and mucosal barrier disruptions in chronic rhinosinusitis. *Immune Netw*. 2017 Feb;17(1):60-7.
45. Nagarkar DR, Poposki JA, Tan BK, Comeau MR, Peters AT, Hulse KE, et al. Thymic stromal lymphopoietin activity is increased in nasal polyps of patients with chronic rhinosinusitis. *J Allergy Clin Immunol*. 2013 Sep;132(3):593-600.e12.
46. Wang BF, Cao PP, Norton JE, Poposki JA, Klingler AI, Suh LA, et al. Evidence that oncostatin M synergizes with IL-4 signaling to induce TSLP expression in chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2023 May;151(5):1379-90.e11.
47. Morshed M, Yousefi S, Stöckle C, Simon HU, Simon D. Thymic stromal lymphopoietin stimulates the formation of eosinophil extracellular traps. *Allergy*. 2012 Sep;67(9):1127-37.
48. Bae CH, Choi YS, Song SY, Kim YD. Effect of thymic stromal lymphopoietin on MUC5B expression in human airway epithelial cells. *Biochem Biophys Res Commun*. 2014 May;448(2):231-5.
49. Datta A, Alexander R, Sulikowski MG, Nicholson AG, Maher TM, Scotton CJ, et al. Evidence for a functional thymic stromal lymphopoietin signaling axis in fibrotic lung disease. *J Immunol*. 2013 Nov;191(9):4867-79.
50. Jin A, Tang X, Zhai W, Li Y, Sun Q, Liu L, et al. Tslp-induced collagen type-I synthesis through Stat3 and Prmt1 is sensitive to calcitriol in human lung fibroblasts. *Biochim Biophys Acta Mol Cell Res*. 2021 Sep;1868(10):119083.
51. Wang ZC, Yao Y, Wang N, Liu JX, Ma J, Chen CL, et al. Deficiency in interleukin-10 production by M2 macrophages in eosinophilic chronic rhinosinusitis with nasal polyps. *Int Forum Allergy Rhinol*. 2018 Nov;8(11):1323-33.
52. Pothoven KL, Norton JE, Hulse KE, Suh LA, Carter RG, Rocci E, et al. Oncostatin M promotes mucosal epithelial barrier dysfunction, and its expression is increased in patients with eosinophilic mucosal disease. *J Allergy Clin Immunol*. 2015 Sep;136(3):737-46.e4.
53. Yuan Y, Zhang Q, Wu B, Huang T, Gong P, Xiang L, et al. Oncostatin M regulates macrophages polarization in osseointegration via yes-associated protein. *Int Immunopharmacol*. 2023 Jul;120:110348.
54. Torossian F, Guerton B, Anginot A, Alexander KA, Desterke C, Soave S, et al. Macrophage-derived oncostatin M contributes to human and mouse neurogenic heterotopic ossifications. *JCI Insight*. 2017 Nov;2(21):e96034.
55. Unver N, McAllister F. Il-6 family cytokines: key inflammatory mediators as biomarkers and potential therapeutic targets. *Cytokine Growth Factor Rev*. 2018 Jun;41:10-7.
56. Headland SE, Dengler HS, Xu D, Teng G, Everett C, Ratsimandresy RA, et al. Oncostatin M expression induced by bacterial triggers drives airway inflammatory and mucus secretion in severe asthma. *Sci Transl Med*. 2022 Jan;14(627):eabf8188.
57. Canè L, Poto R, Palestra F, Pirozzi M, Parashuraman S, Iacobucci I, et al. Tslp is localized in and released from human lung macrophages activated by T2-high and T2-low stimuli: relevance in asthma and COPD. *Eur J Intern Med*. 2024 Jun;124:89-98.
58. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. *J Pathol*. 2013 Jan;229(2):176-85.
59. Chen B, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA. Profiling tumor infiltrating immune cells with CIBERSORT. *Methods Mol Biol*. 2018 Jan;1711:243-59.

Supplementary Table 1. Demographic characteristics of subjects who provided bulk RNA sequencing data

Demographic variable	Control (n=4)	Non-type 2 CRSwNP (n=4)	Type 2 CRSwNP (n=4)	P-value
Age (yr)	48.50±17.62	47.00±18.06	44.50±13.67	0.943
Sex (male)	1 (25.0)	2 (50.0)	3 (75.0)	0.323
Blood eosinophil (cells/ μ L)	184.82±54.97	131.85±53.37	451.73±53.09	<0.001

Values are presented as mean±standard deviation or number (%).

CRSwNP, chronic rhinosinusitis with nasal polyps.

Supplementary Table 2. Demographic characteristics of subjects who provided the *ex vivo* and *in vitro* IL-4 and SEB stimulation study data

Demographic variable	Control (n=4)	Non-type 2 CRSwNP (n=6)	Type 2 CRSwNP (n=6)	P-value
Age (yr)	47.50±24.89	51.83±13.32	57.17±8.73	0.628
Sex (male)	2 (50.0)	4 (66.67)	5 (83.3)	0.532
Blood eosinophil count	150.66±132.88	154.92±26.76	337.03±64.65	0.002
Comorbid asthma	0	1 (16.7)	4 (66.7)	0.052

Values are presented as mean±standard deviation or number (%).

IL, interleukin; SEB, staphylococcal enterotoxin B; CRSwNP, chronic rhinosinusitis with nasal polyps.

Supplementary Table 3. Demographic characteristics of subjects who provided the *ex vivo* and *in vitro* LPS stimulation study data

Demographic variable	Control (n=2)	Non-type 2 CRSwNP (n=8)	Type 2 CRSwNP (n=13)	P-value
Age (yr)	66.50±3.54	54.75±15.61	48.15±10.62	0.138
Sex (male)	0	6 (75.0)	10 (76.9)	0.081
Blood eosinophil count	59.89 (47.79–72.00)	98.06 (66.77–199.93)	452.40 (330.96–620.16)	<0.001
Comorbid asthma	0	0	6 (46.2)	0.044

Values are presented as mean±standard deviation, number (%), or median (interquartile range).

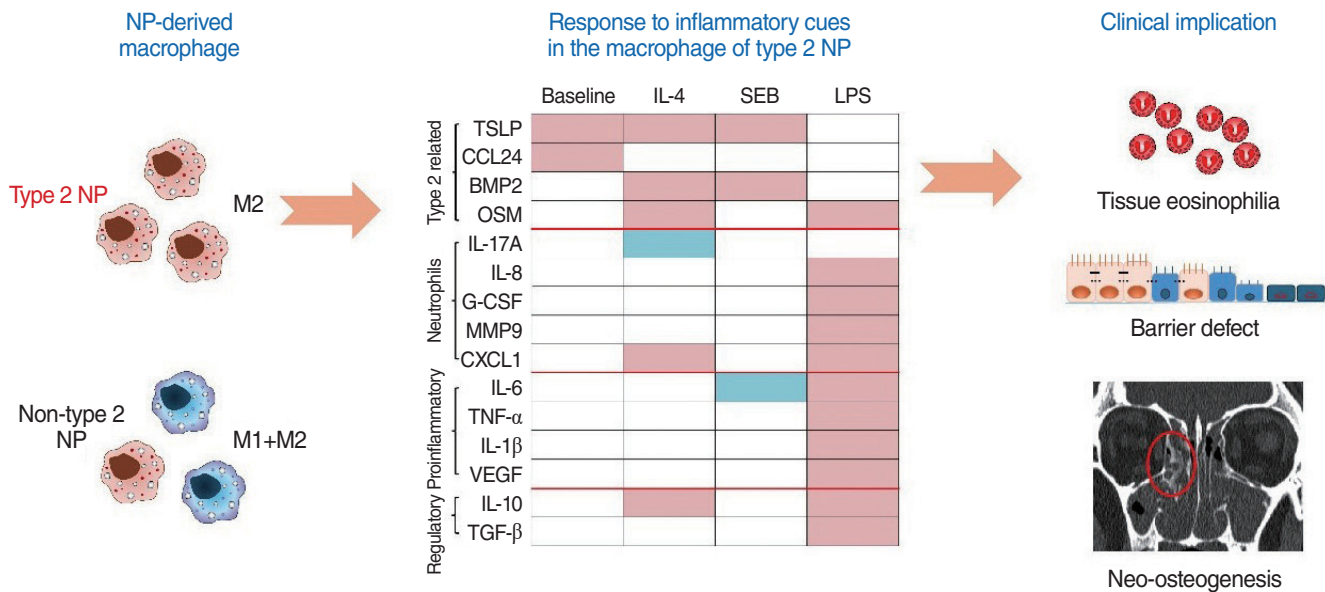
LPS, lipopolysaccharide; CRSwNP, chronic rhinosinusitis with nasal polyps.

Supplementary Table 4. Demographic characteristics of subjects who provided data on TSLP and OSM expression in CD163⁺ cells

Demographic variable	Control (n=5)	Non-type 2 CRSwNP (n=10)	Type 2 CRSwNP (n=10)	P-value
Age (yr)	54.80±18.35	51.70±16.88	51.50±13.10	0.921
Sex (male)	3 (60.0)	9 (90.0)	8 (80.0)	0.392
Tissue eosinophil (/HPF)	-	5.85 (1.00–11.30)	138.20 (108.00–195.60)	<0.001
Tissue neutrophil (/HPF)	-	50.04±24.83	45.70±34.23	0.749
Blood eosinophil count	180.89±105.33	158.94±64.92	595.38±288.61	<0.001
Postoperative 1-yr outcome	-	3 (30.0)	10 (100.0)	0.005
Controlled		7 (70.0)	0	0.002
Partly controlled		0	5 (50.0)	
Uncontrolled		3 (30.0)	5 (50.0)	

Values are presented as mean±standard deviation, number (%), or median (interquartile range).

TSLP, thymic stromal lymphopoietin; OSM, oncostatin M; CRSwNP, chronic rhinosinusitis with nasal polyps; HPF, high power field.



Supplementary Fig. 1. Graphical summary. NP, nasal polyp; IL, interleukin; SEB, staphylococcal enterotoxin B; LPS, lipopolysaccharide; TSLP, thymic stromal lymphopoietin; CCL24, C-C motif chemokine ligand 24; BMP2, bone morphogenetic protein 2; OSM, oncostatin M; G-CSF, granulocyte colony-stimulating factor; MMP9, matrix metalloproteinase 9; CXCL, chemokine (C-X-C motif) ligand; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.