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Phenotypic Analysis of Nasal CD69$^+$ CD4$^+$ Tissue-Resident Memory T Cells in Eosinophilic Chronic Rhinosinusitis With Nasal Polyps

Min-Seok Rha, MD, PhD[1,2], Gyeongyeob Kim, MS[1], and Sol Lee, BS[1]

[1]Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Republic of Korea
[2]The Airway Mucus Institute, Yonsei University College of Medicine, Seoul, Republic of Korea

Background and Objectives: Chronic rhinosinusitis (CRS) is a heterogeneous inflammatory disease, classified into eosinophilic and non-eosinophilic subtypes. Although CD4$^+$ tissue-resident memory T (T_{RM}) cells play diverse roles in tissue homeostasis, their phenotypic and functional characteristics in the nasal tissue of patients with eosinophilic CRS (ECRS) remain poorly defined.

Methods: Nasal polyp tissue and/or peripheral blood (PB) samples were obtained from patients with ECRS undergoing endoscopic sinus surgery. The phenotypes and functions of nasal CD69$^+$ CD4$^+$ T cells were analyzed using flow cytometry.

Results: The frequency of CD69$^+$CD103$^-$ cells among nasal CD4$^+$ T cells was significantly higher in patients with ECRS compared to controls. Analysis of paired PB and nasal tissue samples from ECRS patients revealed that CD69$^+$CD103$^-$ CD4$^+$ T cells were almost exclusively present in nasal tissues and exhibited tissue-resident phenotypes, marked by high expression of CD49a and CXCR6. In ECRS, these nasal CD69$^+$CD103$^-$ CD4$^+$ T cells expressed high levels of T helper 2 (Th2) cell markers, including CRTH2 and GATA3. Consistently, these T cells demonstrated a robust capacity to produce IL-4 and IL-5. These findings were corroborated by analyses of publicly available single-cell RNA sequencing datasets. Furthermore, the frequency of nasal CD69$^+$CD103$^-$ CD4$^+$ T cells was significantly associated with higher Lund-Mackay CT scores and reduced olfactory function in patients with ECRS.

Conclusion: The current investigation demonstrates that nasal CD69$^+$CD103$^-$ CD4$^+$ T_{RM} cells include a high frequency of Th2 cells and are associated with severe disease.

Keywords: Sinusitis; CD4-positive T-lymphocytes; Th2 cells; Memory T cells.

INTRODUCTION

Chronic rhinosinusitis (CRS) is a highly prevalent inflammatory condition of the nasal and paranasal sinus mucosa. The principal symptoms of CRS include facial pain or pressure, congestion, nasal discharge, and hyposmia or anosmia, all of which significantly impair quality of life [1]. CRS is broadly categorized into two phenotypes: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) [1]. Recently, the recognition of inflammatory heterogeneity has prompted a shift in the treatment paradigm of CRS, with increasing focus on the underlying mucosal inflammation [2,3]. Among the diverse immune cell types implicated in CRS, T cells play a central role in its pathogenesis [1,2,4]. Eosinophilic CRS (ECRS) is defined by the presence of abundant T helper (Th) 2 cells producing type 2 (T2) cytokines within nasal polyp (NP) tissue, while non-eosinophilic CRS (NECRS) is characterized by a higher frequency of Th1 and Th17 cells [2,4].

Tissue-resident memory T (T_{RM}) cells are non-migratory memory T cells that reside in non-lymphoid tissues, providing protection against invading pathogens and contributing to tissue homeostasis [5,6]. While the characteristics of CD8$^+$ T_{RM} cells have been extensively studied, the properties of CD4$^+$ T_{RM} cells remain less well understood. In humans, CD4$^+$ T_{RM} cells typically express high levels of CD69 but are less likely than CD8$^+$ T_{RM} cells to express CD103. A notable feature of CD4$^+$ T_{RM} cells is their ability to differentiate into multiple T helper (Th) cell subsets, potentially influenced by the inflam-

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Address for correspondence: Min-Seok Rha, MD, PhD, Department of Otorhinolaryngology, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea
Tel: +82-2-228-3610, **Fax:** +82-2-393-0580, **E-mail:** msrha@yuhs.ac

matory milieu or the specific cognate antigen encountered [7,8]. Our group recently reported that $CD69^+CD103^+$ nasal $CD4^+$ T_{RM} cells may contribute to disease pathogenesis in NECRS by producing IL-17A [9]. However, the phenotypes and functional roles of nasal $CD4^+$ T_{RM} cells in ECRS remain largely unexplored.

In the present study, we collected NP samples from patients with ECRS and analyzed the phenotypes of nasal $CD4^+$ T cells, with a particular focus on $CD69^+$ $CD4^+$ T cell populations. We observed an enrichment of $CD69^+CD103^-$ $CD4^+$ T_{RM} cells within NP tissue from patients with ECRS. Furthermore, we demonstrated that the $CD69^+CD103^-$ $CD4^+$ T_{RM} cell population contains a high frequency of Th2 cells and is associated with increased disease severity in ECRS.

METHODS

Study subjects and sample collection

A total of 47 patients with ECRS who underwent endoscopic sinus surgery (ESS) at Severance Hospital, Korea, between July 2021 and August 2023, were included in this study. The inclusion criteria were: 1) classification as ECRS based on an eosinophil count greater than 10% of inflammatory cells [10,11] and 2) patients who underwent ESS. The exclusion criteria were: 1) presence of fungal ball, antrochoanal polyps, or other autoimmune diseases; 2) use of systemic, topical, or intranasal corticosteroids within four weeks prior to tissue collection; or 3) use of biologics or immunosuppressants. The impact of symptoms on patients' quality of life was assessed using the 22-item Sino-Nasal Outcome Test (SNOT-22). Olfactory function was evaluated with the YSK Olfactory Function (YOF) test (Kimex Co.) [12], which consists of three subtests: threshold (T), discrimination (D), and identification (I). The total TDI score is calculated as the sum of the scores from the three subtests and ranges from 1 to 36.

NP tissue and/or peripheral blood (PB) samples were collected from the patients. In NP tissue samples stained with hematoxylin and eosin, eosinophil counts were quantified in five high-power fields (HPFs) at ×400 magnification. Ethmoid mucosa tissue samples were also obtained from 11 control individuals without evidence of CRS during other nasal surgeries, such as endoscopic skull base surgery or removal of mucous retention cysts in the maxillary or sphenoid sinus. The study was approved by the institutional review boards of Severance Hospital (No. 4-2021-0573). Informed consent was obtained from all study participants, and the study was conducted in compliance with the principles of the Declaration of Helsinki.

Single cell isolation

As described in previous studies [9,13,14], PB mononuclear cells (PBMCs) and nasal cells were isolated from PB and NP tissues, respectively, for flow cytometry analysis. Briefly, PBMCs were obtained via density gradient centrifugation using Lymphocyte Separation Medium (Corning). Nasal single-cell suspensions were prepared by mechanical dissociation of NP tissues, followed by enzymatic digestion with the Tumor Dissociation Kit (Miltenyi Biotec) in combination with a gentleMACS dissociator (Miltenyi Biotec).

Flow cytometry

Cells were incubated with fluorochrome-conjugated antibodies at room temperature for 10 minutes to stain surface markers. Non-viable cells were excluded using LIVE/DEAD near-IR dye (Invitrogen). For intracellular staining, cells were fixed and permeabilized using the FoxP3 transcription factor staining buffer kit (Thermo Fisher Scientific), followed by staining for intracellular markers for 30 minutes at 4°C. Multiparameter flow cytometry was performed using either a Fortessa or Lyric cytometer (BD Biosciences), and data were processed and analyzed with FlowJo software version 10.10 (FlowJo LLC).

The following antibodies were obtained from BD Biosciences: anti-CXCR6 BV421 (clone 13B 1E5), anti-CD3 BV510 (clone UCHT1), anti-CD8 BV605 (clone SK1), anti-CD69 BV786 (clone FN50), anti-CD45RA BB515 (clone HI100), anti-CCR7 PerCP-Cy5.5 (clone 150503), anti-CD4 Alexa Fluor 700 (clone RPA-T4), anti-CD14 APC-Cy7 (clone MφP9), anti-CD19 APC-Cy7 (clone SJ25C1), anti-IL-5 APC (clone TRFK5), anti-CRTH2 APC-Cy7 (clone SJ25C1), and anti-GATA3 BV421 (clone L50-823). The following antibodies were from BioLegend: anti-CD103 PE (clone Ber-ACT8), anti-CD49a PE-Cy7 (clone TS2/7), and anti-IL-4 PE/Dazzle 594 (clone MP4-25D2).

In vitro stimulation

For intracellular cytokine staining, nasal cells were stimulated with 10 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) and 500 ng/mL ionomycin (Sigma-Aldrich) for 4 hours, in combination with the protein transport inhibitor brefeldin A (GolgiPlug, BD Biosciences) and monensin (GolgiStop, BD Biosciences). After stimulation, cells were harvested and analyzed by flow cytometry.

Analysis of single-cell RNA sequencing data

Single-cell RNA sequencing (scRNA-seq) data from a publicly available dataset (Genome Sequence Archive HRA000772) were analyzed [15], focusing on samples from control individuals and patients with CRSwNP. In the referenced study, pa-

tients were classified as having ECRS if their tissue eosinophil counts were ≥10 per HPF. As described previously [9], raw gene expression matrices were processed using the Cell Ranger pipeline (version 7.1.0; 10× Genomics), with the GRCh38 human genome as reference. The resulting gene-cell unique molecular identifier matrix was analyzed using R (version 4.3.0; R Foundation for Statistical Computing) and the Seurat package (version 4.1.3). For quality control, cells were excluded if they expressed mitochondrial genes in more than 15% of their transcriptome, expressed fewer than 200 genes, or more than 10,000 genes. Normalization was performed based on total transcript counts per cell. The dataset was then scaled (ScaleData function), and principal component analysis (PCA) was performed to reduce dimensionality (RunPCA function). Unsupervised clustering was conducted using the FindClusters function (resolution=0.2), and data were visualized using t-distributed stochastic neighbor embedding with the top 20 principal components (RunTSNE). To further analyze the "T cell" population in detail, uniform manifold approximation and projection (UMAP) was applied using the top 20 PCs, followed by unsupervised clustering (resolution=0.2, FindClusters function). The CD4$^+$ T cell subset was further analyzed by subclustering via UMAP with the top 25 PCs (resolution=0.4, FindClusters function).

Statistical analysis

All statistical analyses were performed using GraphPad Prism software (version 9.1.1), with statistical significance defined as $p<0.05$. The Wilcoxon signed-rank test was used to compare paired groups, and the Mann–Whitney U test was used for unpaired group comparisons. Differences among multiple groups were assessed with one-way repeated-measures analysis of variance followed by the Tukey multiple comparisons test. The Pearson correlation test was used to assess the significance of correlations.

RESULTS

CD69$^+$CD103$^-$ CD4$^+$ T_{RM} cells are enriched in the nasal tissue of patients with ECRS

In this study, 47 patients with ECRS were included. The demographic and clinical characteristics of the patients are summarized in Table 1. We first analyzed the expression of CD69 and CD103, canonical markers of T_{RM} cells [7,8], in nasal CD4$^+$ T cells (Fig. 1A). The frequency of CD69$^+$CD103$^-$ cells among nasal CD4$^+$ T cells was significantly higher in the ECRS group than in the control group (Fig. 1B), while the frequencies of CD69$^-$CD103$^-$ and CD69$^+$CD103$^+$ cells did not differ between groups.

When we analyzed paired PBMC and NP samples from patients with ECRS, both CD69$^+$CD103$^-$ and CD69$^+$CD103$^+$ cells were detected exclusively among CD4$^+$ T cells in NP tissues, but not in PB (Fig. 2A). In addition, nasal CD69$^+$CD103$^-$ and CD69$^+$CD103$^+$ CD4$^+$ T cells expressed significantly higher levels of tissue-residency markers, including CD49a and CXCR6, compared to nasal CD69$^-$CD103$^-$ CD4$^+$ T cells (Fig. 2B). These results indicate that nasal CD69$^+$CD103$^-$ and CD69$^+$CD103$^+$ CD4$^+$ T cells exhibit tissue-resident characteristics. We further examined the differentiation status of nasal CD69$^-$CD103$^-$, CD69$^+$CD103$^-$, and CD69$^+$CD103$^+$ CD4$^+$ T-cell subsets according to CCR7 and CD45RA expression. The majority of nasal CD69$^+$CD103$^-$ and CD69$^+$CD103$^+$ CD4$^+$ T cells were effector memory T (CCR7$^-$CD45RA$^-$; T_{em}) cells, whereas only a small proportion were naïve T (CCR7$^+$CD45RA$^+$; $T_{naïve}$) cells (Fig. 2C). Collectively, these findings demonstrate that CD69$^+$CD103$^-$ CD4$^+$ T cells with tissue-resident memory features are enriched in the NP tissue of patients with ECRS.

Table 1. Demographic and clinical characteristics of the study subjects

Parameter	Control (n=11)	ECRS (n=47)
Tissue used	Ethmoid mucosa mucosa	NP
Age (yr)	53 (40.5–65.5)	51 (39.0–59.5)
Sex		
Female	7 (63.6)	13 (27.7)
Male	4 (36.4)	34 (72.3)
Lund-Mackay CT score	N/A	16 (12–21)
Asthma	0 (0)	10 (23.4)

Data are presented as median (interquartile range) or number (%).
ECRS, eosinophilic chronic rhinosinusitis; NP, nasal polyp; CT, computed tomography; N/A, not available

Nasal CD69$^+$CD103$^-$ CD4$^+$ T cells express high levels of CRTH2 and GATA3 in ECRS

Th2 cells are known to play a central role in the pathophysiology of ECRS [2]. Therefore, we further assessed the expression of Th2-associated markers in nasal CD4$^+$ T cells according to CD69 and CD103 expression. Because the prostaglandin D2 receptor CRTH2 serves as a marker for identifying Th2 cells in humans [16], we first analyzed the frequency of CRTH2$^+$ cells among nasal CD4$^+$ T cells in relation to CD69 and CD103 expression. We found that the frequency of CRTH2$^+$ cells was significantly higher in the CD69$^+$CD103$^-$ CD4$^+$ T-cell subset than in the CD69$^-$CD103$^-$ and CD69$^+$CD103$^+$ CD4$^+$ T-cell subsets (Fig. 3A). Consistent with this, the expression level of GATA3—the master transcription factor for Th2 cells [17]—was also significantly higher in the CD69$^+$CD103$^-$ CD4$^+$ T-cell subset compared to the other two subsets (Fig. 3B).

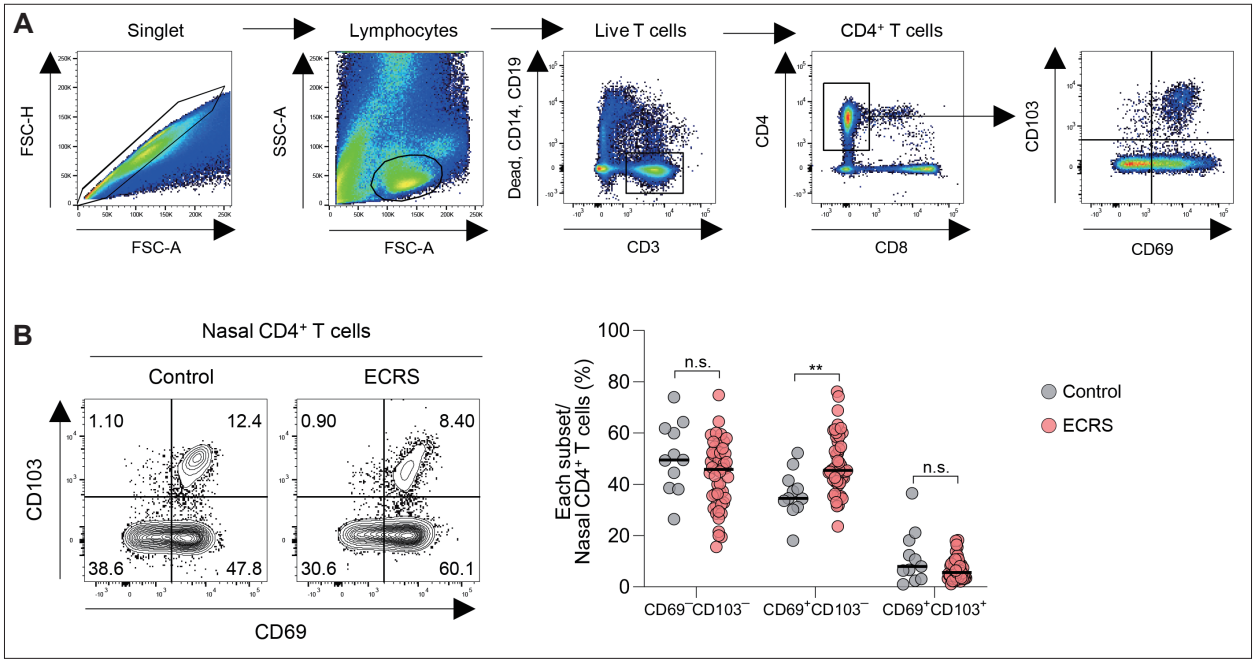

Fig. 1. Frequency of CD69$^+$ cells among nasal CD4$^+$ T cells in control individuals and patients with ECRS. A: The flow cytometry gating strategy used to analyze the expression of CD69 and CD103 on nasal CD4$^+$ T cells. B: Representative plots and summary data illustrating the frequency of the indicated cell subsets among nasal CD4$^+$ T cells in control individuals (n=11) and patients with ECRS (n=47). Statistical analysis was conducted using the Mann–Whitney U test (B). **p<0.01. ECRS, eosinophilic chronic rhinosinusitis; n.s., not significant.

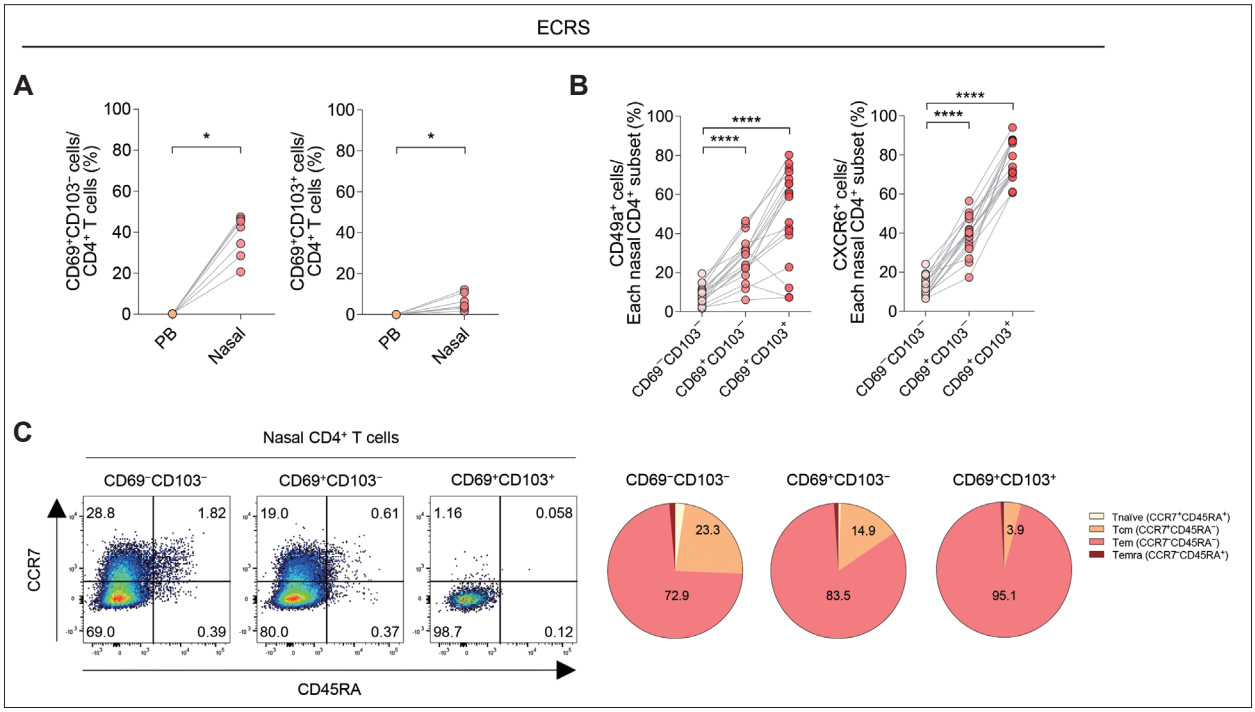

Fig. 2. Characteristics of nasal CD4$^+$ T cells according to the expression of CD69 and CD103 in patients with ECRS. PBMCs and nasal cells from patients with ECRS were analyzed. A: The frequencies of CD69$^+$CD103$^-$ and CD69$^+$CD103$^+$ cells among CD4$^+$ T cells from paired PBMCs and nasal cells (n=7). B: The frequencies of CD49a$^+$ and CXCR6$^+$ cells among the indicated nasal CD4$^+$ T-cell subsets (n=18). C: The frequencies of naïve T (CCR7$^+$CD45RA$^+$; Tnaïve), central memory T (CCR7$^+$CD45RA$^-$; Tcm), effector memory T (CCR7$^-$CD45RA$^-$; Tem), terminally differentiated effector memory T (CCR7$^-$CD45RA$^+$; Temra) cells in the indicated nasal CD4$^+$ T-cell subsets (n=12). Statistical analyses were conducted using the Wilcoxon signed-rank test (A) or one-way repeated-measures ANOVA with the Tukey multiple comparisons test (B). *p<0.05; ****p<0.0001. ECRS, eosinophilic chronic rhinosinusitis; PBMC, peripheral blood mononuclear cell; ANOVA, analysis of variance.

Nasal CD69$^+$CD103$^-$ CD4$^+$ T cells exhibit a robust capacity to produce type 2 cytokines in ECRS

Next, we investigated the functional profiles of nasal CD4$^+$ T cells from patients with ECRS based on CD69 and CD103 expression. The capacity to produce T2 cytokines, including IL-4 and IL-5, following ex vivo stimulation was assessed. We

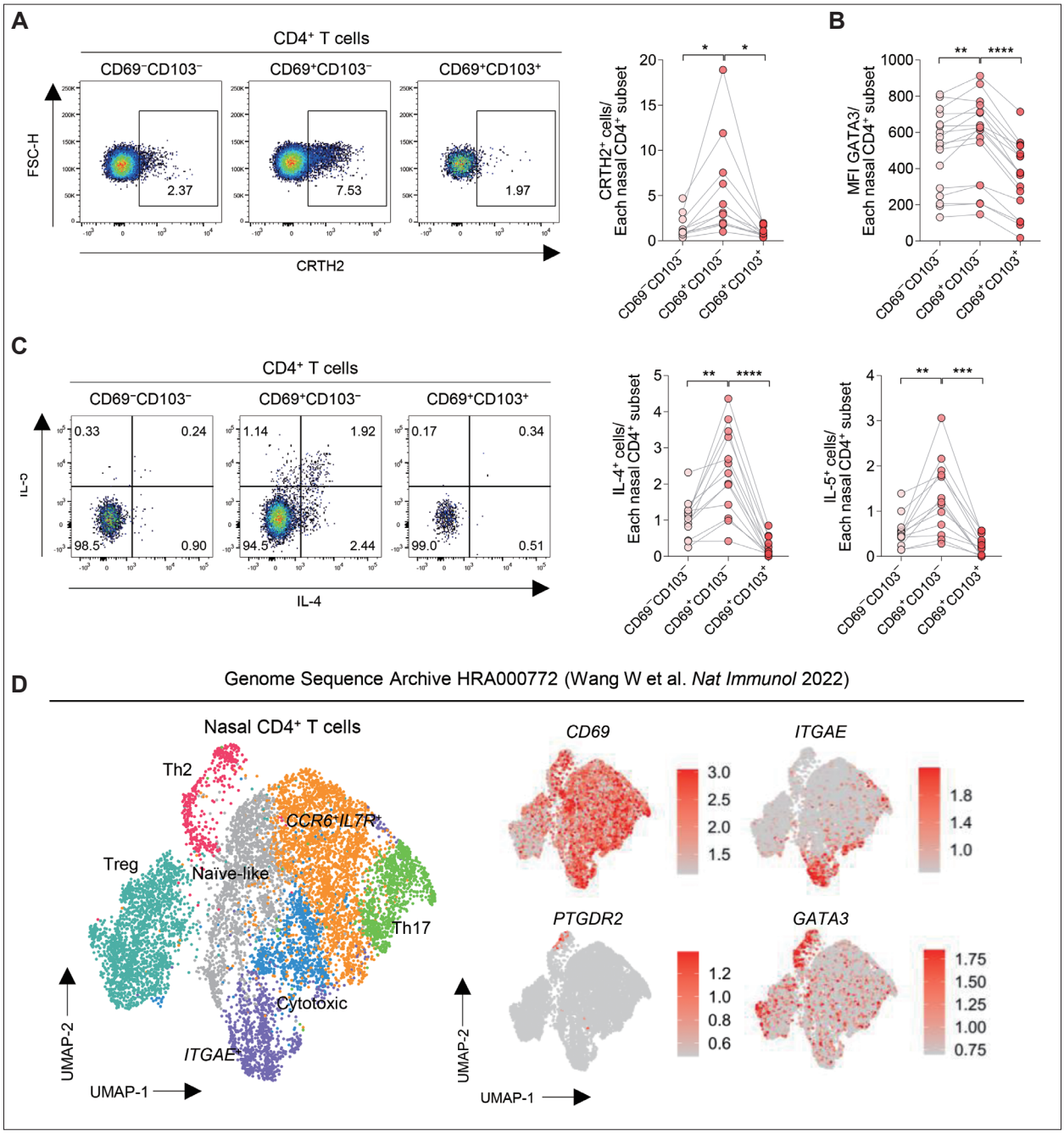

Fig. 3. Th2 phenotype and function of nasal CD4$^+$ T cells according to the expression of CD69 and CD103 in patients with ECRS. A: Representative plots and summary data illustrating the frequencies of CRTH2$^+$ cells among the indicated nasal CD4$^+$ T-cell subsets (n=11). B: MFI of GATA3 in the indicated nasal CD4$^+$ T-cell subsets (n=17). C: Nasal cells from patients with ECRS were stimulated with PMA and ionomycin for 4 hours and analyzed for intracellular cytokine expression. Frequencies of IL-4$^+$ and IL-5$^+$ cells among the indicated nasal CD4$^+$ T-cell subsets (n=13). D: Publicly available scRNA-seq data were analyzed [15]. UMAP projections of 9,623 nasal CD4$^+$ T cells showing the expression of *CD69*, *ITGAE*, *PTGDR2*, and *GATA3*. Statistical analyses were conducted using one-way repeated-measures ANOVA with the Tukey multiple comparisons test (A-C). *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. MFI, mean fluorescence intensity; IL, interleukin; scRNA-seq, single-cell RNA sequencing; UMAP, uniform manifold approximation and projection; ANOVA, analysis of variance.

observed that CD69$^+$CD103$^-$ CD4$^+$ T cells produced significantly higher levels of IL-4 and IL-5 compared to CD69$^-$ CD103$^-$ and CD69$^+$CD103$^+$ CD4$^+$ T cells (Fig. 3C). Supporting these results, analysis of scRNA-seq data from a publicly accessible dataset [15] revealed that the majority of the Th2 cell cluster—characterized by expression of *PTGDR2* (encoding CRTH2) and *GATA3* transcripts—expressed CD69 but rarely expressed *ITGAE* (encoding CD103) (Fig. 3D). Together, these results indicate that nasal CD69$^+$CD103$^-$ CD4$^+$ T cells harbor a high frequency of Th2 cells in ECRS.

The frequency of nasal CD69$^+$CD103$^-$ CD4$^+$ T cells correlates with disease severity and olfactory impairment in patients with ECRS

Lastly, we assessed the clinical relevance of nasal CD69$^+$ CD103$^-$ CD4$^+$ T cells in patients with ECRS. The frequency of CD69$^+$CD103$^-$ cells—but not CD69$^+$CD103$^+$ cells—among nasal CD4$^+$ T cells showed a significant correlation with disease extent, as assessed by the Lund-Mackay CT score (Fig. 4A). These results suggest that enrichment of CD69$^+$CD103$^-$ CD4$^+$ T cells in NP tissue is more pronounced in severe disease. SNOT-22 scores were not correlated with the frequency of either CD69$^+$CD103$^-$ or CD69$^+$CD103$^+$ nasal CD4$^+$ T cells (Fig. 4B). Interestingly, TDI scores from psychophysical olfactory function tests showed a significant inverse correlation with the frequency of CD69$^+$CD103$^-$ CD4$^+$ T cells, but not with that of CD69$^+$CD103$^+$ CD4$^+$ T cells (Fig. 4C). However, no significant correlations were observed between the frequencies of either nasal CD69$^+$CD103$^-$ or CD69$^+$CD103$^+$ CD4$^+$ T cells and blood or tissue eosinophil counts (Fig. 4D and E). These findings suggest that the relationship between Th2 cells and eosinophilic inflammation is highly complex. Specifically, the de-

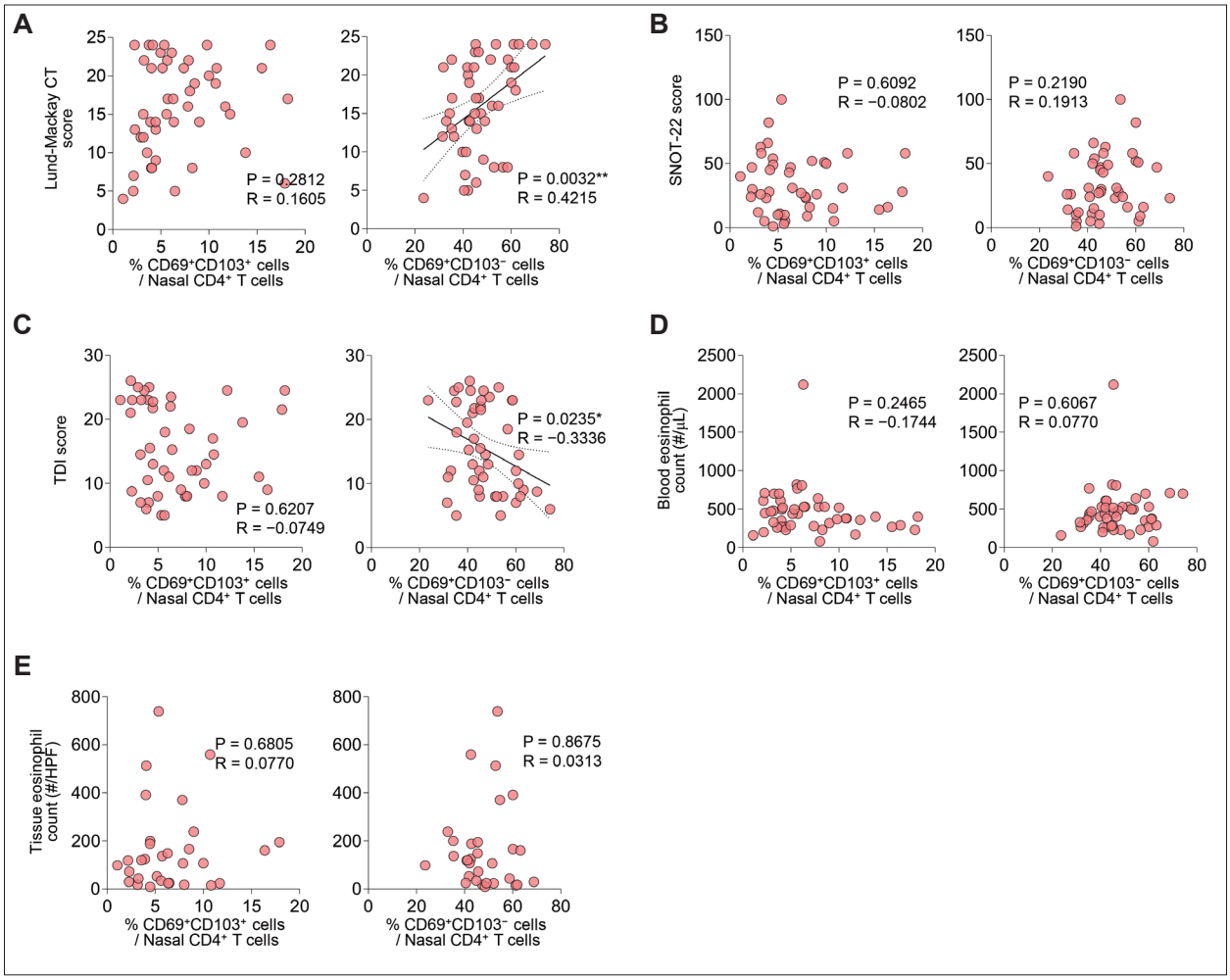

Fig. 4. Correlation analysis of the frequencies of nasal CD69$^+$CD103$^-$ and CD69$^+$CD103$^+$ CD4$^+$ T cells with various clinical variables. Correlation analysis between the frequency of CD69$^+$CD103$^-$ or CD69$^+$CD103$^+$ cells among nasal CD4$^+$ T cells and the following clinical parameters: Lund-Mackay CT score (n=47) (A), SNOT-22 score (n=43) (B), TDI score (n=46) (C), blood eosinophil counts (n=47) (D), or tissue eosinophil counts (n=31) (E). Statistical analyses were conducted using the Pearson correlation test. *p<0.05; **p<0.01. CT, computed tomography; SNOT-22, 22-item Sino-Nasal Outcome Test; TDI, threshold-discrimination-identification.

gree of eosinophilic inflammation may not be solely determined by the frequency of Th2 cells. Other immune cells, such as type 2 innate lymphoid cells and mast cells, also contribute to eosinophilic inflammation through the production of T2 cytokines in patients with ECRS.

DISCUSSION

CRS is a highly heterogeneous disease with a wide range of clinical manifestations and inflammatory patterns. An in-depth understanding of the immunopathogenesis of CRS would facilitate precision medicine and improve patient care. In the current study, we demonstrated that nasal CD69$^+$CD103$^-$ CD4$^+$ T cells are more abundant in patients with ECRS compared to control individuals. Notably, this nasal CD4$^+$ T-cell subset highly expresses key Th2 markers, including CRTH2 and GATA3, and possesses a robust capacity to produce T2 cytokines. Furthermore, the enrichment of nasal CD69$^+$CD103$^-$ CD4$^+$ T cells correlates with disease extent, suggesting their contribution to disease development. Our findings provide new insights into the potential role of nasal CD4$^+$ T_{RM} cells in ECRS.

T_{RM} cells are considered key players in tissue homeostasis and disease. Owing to their memory features, T_{RM} cells rapidly exert effector functions upon antigen re-encounter [8,18], thereby contributing to host immune defense. In contrast, pathogenic roles of T_{RM} cells have been reported in inflammatory conditions, such as psoriasis and inflammatory bowel disease [19]. However, knowledge regarding nasal T_{RM} cells is currently limited. We previously reported potential pathogenic roles of CD69$^+$CD103$^+$ CD4$^+$ T_{RM} cells in NECRS [9]. In the present study, we analyzed the frequency and phenotypes of CD69$^+$CD103$^-$ CD4$^+$ T cells in the context of ECRS. We found that nasal CD69$^+$CD103$^-$ CD4$^+$ T cells highly expressed CD49a and CXCR6 in patients with ECRS, implying that these T cells exhibit tissue-resident characteristics. Additionally, when we analyzed their differentiation status, the majority of nasal CD69$^+$CD103$^-$ CD4$^+$ T cells were effector memory T cells. These findings collectively demonstrate enrichment of CD69$^+$CD103$^-$ CD4$^+$ T cells with tissue-resident memory features in the nasal tissue of patients with ECRS. However, the relatively small sample size of the control group is a limitation of this study and may affect the statistical power and generalizability of our findings. Future studies with larger cohorts are needed to validate these results.

CRTH2 is widely recognized as a reliable marker for Th2 cell identification [16]. Furthermore, GATA3 is a critical regulator of the effector functions and differentiation of Th2 cells [17]. Intriguingly, we observed significantly higher expression levels of CRTH2 and GATA3 in nasal CD69$^+$CD103$^-$ CD4$^+$ T cells compared to their CD69$^+$CD103$^+$ and CD69$^-$CD103$^-$ counterparts. These findings imply that the nasal CD69$^+$CD103$^-$ CD4$^+$ T-cell population harbors a high proportion of pathogenic Th2 cells. Consistent with this, analysis of publicly available scRNA-seq data revealed that the Th2 cell cluster, characterized by *PTGDR2* and *GATA3* expression, highly expressed *CD69* but rarely *ITGAE*. Further studies are needed to investigate the mechanisms underlying the low expression of CD103 in Th2 cells. Given that CD103 is the αE subunit of the αEβ7 integrin, which interacts with E-cadherin [20], investigating the localization of Th2 cells within the NP tissue of patients with ECRS using spatial analysis would be of particular interest. Moreover, using functional assays, we also confirmed that nasal CD69$^+$CD103$^-$ CD4$^+$ T cells robustly produced T2 cytokines.

Furthermore, we found a significant correlation between disease severity and the frequency of nasal CD69$^+$CD103$^-$ CD4$^+$ T cells in patients with ECRS. This association may be attributed to the high frequency of pathogenic Th2 cells within this CD4$^+$ T-cell subset. Given their tissue residency and memory features, persistent pathogenic nasal CD4$^+$ T_{RM} cells with Th2 phenotypes may also contribute to disease recurrence upon exposure to aggravating factors [3]. Additionally, their significant association with impaired olfactory function supports a potential pathogenic role in the pathophysiology of ECRS. Therefore, these CD4$^+$ T_{RM} cells may serve as potential biomarkers for predicting severe disease with olfactory impairment, as well as therapeutic targets for the treatment of ECRS. The regulatory mechanisms of pathogenic nasal CD4$^+$ T_{RM} cells in ECRS warrant further investigation.

In summary, our study revealed the phenotype and function of nasal CD69$^+$CD103$^-$ CD4$^+$ T cells in ECRS, particularly in relation to Th2 cells. We demonstrated that these CD4$^+$ T_{RM} cells harbor a high frequency of Th2 cells and are associated with severe disease. These results provide valuable insights into the potential role of nasal CD4$^+$ T_{RM} cells in the pathogenesis of ECRS.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

Conflicts of Interest

Min-Seok Rha who is on the editorial board of the *Journal of Rhinology* was not involved in the editorial evaluation or decision to publish this article. All remaining authors have declared no conflicts of interest.

Author Contributions

Conceptualization: Min-Seok Rha. **Data curation:** all authors. **Formal analysis:** all authors. **Funding acquisition:** Min-Seok Rha. **Investigation:** all authors. **Methodology:** all authors. **Project administration:** Min-Seok Rha. **Resources:** Min-Seok Rha. **Software:** all authors. **Supervision:** Min-Seok Rha. **Validation:** all authors. **Visualization:** Min-Seok Rha. **Writing—**

original draft: Min-Seok Rha. **Writing—review & editing:** Min-Seok Rha.

ORCID iDs

Min-Seok Rha https://orcid.org/0000-0003-1426-7534
Gyeongyeob Kim https://orcid.org/0000-0002-7972-1446

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